

The Journal of Parasitology

The Official Organ of the American Society of Parasitologists

Founded by HENRY BALDWIN WARD

VOLUME XXVIII
1942

EDITORIAL BOARD

HAROLD W. BROWN

University of North Carolina

ASA C. CHANDLER

Rice Institute

WILLIAM W. CORT

Johns Hopkins University

RICHARD P. HALL

New York University

E. HAROLD HINMAN

Tennessee Valley Authority

HAROLD KIRBY, Jr.

University of California

HAROLD W. MANTER

University of Nebraska

REGINALD D. MANWELL

Syracuse University

JUSTUS F. MUELLER

Syracuse University

CORNELIUS B. PHILIP

U. S. Public Health Service

BENJAMIN SCHWARTZ

U. S. Bureau of Animal Industry

ERNEST E. TYZZER

Harvard University

EDITORIAL COMMITTEE

NORMAN R. STOLL, Chairman

Rockefeller Institute, Princeton, N. J.

WILLIAM H. TALIAFERRO

University of Chicago

WILLIAM A. RILEY

University of Minnesota

THE SCIENCE PRESS PRINTING COMPANY
LANCASTER, PENNSYLVANIA

CONTENTS OF VOLUME 28

FEBRUARY, 1942, NUMBER 1.

J. E. ACKERT, Portrait	Frontispiece
Ackert, J. E. NATURAL RESISTANCE TO HELMINTHIC INFECTIONS	1
Brackett, Sterling. FIVE NEW SPECIES OF AVIAN SCHISTOSOMES FROM WISCONSIN AND MICHIGAN WITH THE LIFE CYCLE OF <i>Gigantobilharsia gyrauli</i> (BRACKETT, 1940)	25
Wood, Sherwin F. REACTIONS OF MAN TO THE FEEDING OF REDUVIID BUGS ..	43
Byrd, Elon E. and Robert J. Reiber. STRIGEID TREMATODES OF THE ALLIGATOR, WITH REMARKS ON THE PROSTATE GLAND AND TERMINAL PORTIONS OF THE GENITAL DUCTS	51
Olivier, Louis and W. W. Cort. AN EXPERIMENTAL TEST OF THE LIFE CYCLE DESCRIBED FOR <i>Cotylurus communis</i> (HUGHES)	75
Meglitsch, Paul A. ON TWO NEW SPECIES OF MYXOSPORIDIA FROM ILLINOIS FISHES	83
RESEARCH NOTES.	
Rothschild, Miriam. A FURTHER NOTE ON LIFE HISTORY EXPERIMENTS WITH <i>Cryptocotyle lingua</i> (CREPLIN, 1825)	91
Ishii, Nobutaro. NEW PARASITE RECORDS FROM THE RUFFED GROUSE	92
Wallace, F. G. A DEVICE FOR ADMINISTERING PARTICULATE MATERIAL TO MICE	92
Meleney, Henry E. THE DURATION OF HUMAN INFECTION WITH <i>Endamoeba histolytica</i> AND OTHER INTESTINAL PROTOZOA	93
Hawkins, Philip A. <i>Sigmodon hispidus hispidus</i> , A NEW HOST FOR THE STROBILOCERCUS OF <i>Taenia taeniaeformis</i>	94

APRIL, 1942, NUMBER 2.

Sawitz, Willi. THE BUOYANCY OF CERTAIN NEMATODE EGGS	95
Penner, Lawrence R. STUDIES ON DERMATITIS-PRODUCING SCHISTOSOMES IN EASTERN MASSACHUSETTS, WITH EMPHASIS ON THE STATUS OF <i>Schistosomatium pathlocopticum</i> TANABE, 1923	103
Harkema, Reinard. <i>Pharyngostomoides procyonis</i> N.G., N. SP. (STRIGEIDA), A TREMATODE FROM THE RACCOON IN NORTH CAROLINA AND TEXAS	117
Glaser, R. W., E. E. McCoy and H. B. Girth. THE BIOLOGY AND CULTURE OF <i>Neoapectana chresima</i> , A NEW NEMATODE PARASITIC IN INSECTS	123
Russell, Paul F. and Badri Nath Mohan. SOME MOSQUITO HOSTS TO AVIAN PLASMODIA WITH SPECIAL REFERENCE TO <i>Plasmodium gallinaceum</i>	127
Butler, Robert L., Jr. and Reed O. Christenson. A SIMPLE APPARATUS FOR DETERMINING THE VIABILITY OF EMBRYONATED HELMINTH OVA	131
Chandler, Asa C. HELMINTHS OF TREE SQUIRRELS IN SOUTHEAST TEXAS	135
Polk, Seigul J. <i>Hymenolepis mastigopraedita</i> , A NEW CESTODE FROM A PINTAIL DUCK	141
Osimani, J. J. <i>Haemogregarina triatomae</i> N. SP. FROM A SOUTH AMERICAN LIZARD <i>Tupinambis teguixin</i> TRANSMITTED BY THE REDUVIID <i>Triatoma rubrovaria</i>	147
Culbertson, James T. and Walter R. Kessler. AGE RESISTANCE OF MICE TO <i>Trypanosoma cruzi</i>	155
Tucker, Hal. <i>Nematodirus tortuosus</i> N. SP. (NEMATODA) FROM THE RAT, <i>Neotoma</i>	159
RESEARCH NOTES.	
Carvalho, José Candido M. NOTE ON THE ASSOCIATION OF THE TICK, <i>Ornithodoros talaje</i> (GUÉRIN-MENEVILLE), WITH BAT INFESTATIONS IN HUMAN DWELLINGS IN BRAZIL	165

Walton, A. C. <i>Paralaeuris cuckleri</i> N. SP. (NEMATODA) FROM THE IGUANA (<i>Cyclura cornuta</i>)	165
Fischthal, Jacob H. A <i>Paragordius</i> LARVA (GORDIACEA) IN A TREMATODE	167
Huizinga, Henry. <i>Eimeria antelocaprae</i> , A NEW COCCIDIUM FROM THE AMERICAN ANTELOPE	167
Whitlock, J. H. STUDIES UPON <i>Strongylus vulgaris</i> . VI. TESTS WITH ORGANIC COPPER SALTS	168
Summers, William A. INTESTINAL PARASITES IN BOYS OF THE FLORIDA INDUSTRIAL SCHOOL	169
Kartman, Leo. A NOTE ON VITAMINS IN RELATION TO ECTOPARASITE RESISTANCE	170
COMMUNICATIONS.	
Finlay, C. E. CARLOS FINLAY AND YELLOW FEVER	172
Hartwell, Wayne M. CONSERVATION OF SCHOLARLY JOURNALS	174

JUNE, 1942, NUMBER 3.

ROBERT WILLIAM HEGNER (1880-1942)	175
Herber, E. C. LIFE HISTORY STUDIES ON TWO TREMATODES OF THE SUBFAMILY NOTOCOTYLINAE	179
Culbertson, James T. ACTIVE IMMUNITY IN MICE AGAINST <i>Trichinella spiralis</i>	197
Culbertson, James T. PASSIVE TRANSFER OF IMMUNITY TO <i>Trichinella spiralis</i> IN THE RAT	203
Greenfield, Sylvia H. AGE RESISTANCE OF THE ALBINO RAT TO <i>Cysticercus fasciolaris</i>	207
Carvalho, José Candido M. STUDIES ON SOME GORDIACEA OF NORTH AND SOUTH AMERICA	213
Peres, Charles E. <i>Trichinella spiralis</i> . II. INCIDENCE OF INFECTION IN HOGS AND RATS IN THE NEW ORLEANS AREA	223
Chandler, Asa C. <i>Mesocestoides manteri</i> N. SP. FROM A LYNX, WITH NOTES ON OTHER NORTH AMERICAN SPECIES OF <i>Mesocestoides</i>	227
MacDougall, Mary Muriel. A STUDY OF TEMPERATURE EFFECTS ON GRE-GARINES OF <i>Tenebrio molitor</i> LARVAE	233
Herde, Karl E. A NEW SPIRUROID NEMATODE, <i>Thelasia buteonis</i> , FROM SWAINSON'S HAWK	241
RESEARCH NOTES.	
Roth, Hans and N. O. Christensen. OCCURRENCE IN THE HORSE OF TWO PARASITES OF CATTLE, <i>Ostertagia ostertagi</i> (STILES, 1892) AND <i>Cooperia oncophora</i> (RAILLIET, 1898)	245
Wolfson, Fruma. ORAL TRANSMISSION OF <i>Plasmodium cathemerium</i> BY MEANS OF TISSUES	245
Beltran, Enrique and Luis Vargas. THE INTRAVENOUS INOCULATION OF SPOROZOITES OF <i>Plasmodium gallinaceum</i>	246
AMERICAN SOCIETY OF PARASITOLOGISTS.	
SEVENTEENTH ANNUAL MEETING, DALLAS, TEXAS, DECEMBER 29 TO 31, 1941	247
CHANGES IN MEMBERSHIP	250
REPORT OF THE COMMITTEE ON TERMINOLOGY OF STRAINS OF AVIAN MALARIA	250

AUGUST, 1942, NUMBER 4.

Chandler, Asa C. THE HELMINTHS OF RACCOONS IN EAST TEXAS	255
Fischthal, Jacob H. THREE NEW SPECIES OF <i>Phyllodistomum</i> (TREMATODA: GORGODERIDAE) FROM MICHIGAN FISHES	269
Penn, George H., Jr. THE LIFE HISTORY OF <i>Porocephalus crotali</i> , A PARASITE OF THE LOUISIANA MUSKRAT	277
Chen, H. T. THE METACERCARIA AND ADULT OF <i>Centrocestus formosanus</i> (NISHIGORI, 1924), WITH NOTES ON THE NATURAL INFECTION OF RATS AND CATS WITH <i>C. armatus</i> (TANABE, 1922)	285

Bellamy, R. Edward. OBSERVATIONS ON THE MACROSCOPIC SPECIES-IDENTIFICATION OF LARVAL <i>Anopheles</i> IN GEORGIA	299
Kirby, Harold. A PARASITE OF THE MACRONUCLEUS OF <i>Vorticella</i>	311
von Brand, Theodor and Jean Saurwein. FURTHER STUDIES UPON THE CHEMISTRY OF <i>Macracanthorhynchus hirudinaceus</i>	315
Reid, W. M. CERTAIN NUTRITIONAL REQUIREMENTS OF THE FOWL CESTODE <i>Raillietina cesticillus</i> (MOLIN) AS DEMONSTRATED BY SHORT PERIODS OF STARVATION OF THE HOST	319
Spurlock, G. M. and J. T. Emlen, Jr. <i>Hypodectes chapini</i> N. SP. (ACARINA) FROM THE RED-SHAFTED FLICKER	341
RESEARCH NOTES.	
Summers, William A. A MODIFICATION OF ZINC SULFATE CENTRIFUGAL FLOTATION METHOD FOR RECOVERY OF HELMINTH OVA IN FORMALINIZED FECES	345
Levine, P. P. THE PERIODICITY OF OÖCYST DISCHARGE IN COCCIDIAL INFECTION OF CHICKENS	346
Penn, George H., Jr. PARASITOLOGICAL SURVEY OF LOUISIANA MUSKRATS	348
McNeil, E. and W. R. Hinshaw. <i>Cochlosoma rostratum</i> FROM THE TURKEY	349
Rothschild, Miriam. A SEVEN-YEAR-OLD INFECTION OF <i>Cryptocotyle lingua</i> CREPLIN IN THE WINKLE <i>Littorina littorea</i> L.	350

OCTOBER, 1942, NUMBER 5.

Threlkeld, W. L. and M. E. Henderson. NOTES ON THE MUSCULATURE OF THE MALE GENITALIA OF <i>Haemonchus contortus</i>	351
Kohls, Glen M. SIPHONAPTERA: <i>Ptilopssylla dunni</i> , A NEW SPECIES OF BAT FLEA FROM PANAMA	361
DeEds, Floyd and John O. Thomas. STUDIES ON PHENOTHIAZINE. XI. THE EXCRETION OF PHENOTHIAZONE	363
Johnson, Garth. PHYSIOLOGY OF A BACTERIA-FREE CULTURE OF <i>Trichomonas vaginalis</i> . IV. EFFECT OF HYDROGEN ION CONCENTRATION AND OXYGEN TENSION ON POPULATION	369
Goble, Frans C. <i>Crenosoma sederi</i> N. SP. (NEMATODA: METASTRONGYLOIDEA), A NEW LUNGWORM FROM THE SKUNK (<i>Mephitis nigra</i>)	381
Perry, Mary Louise. A NEW SPECIES OF THE ACANTHOCEPHALAN GENUS <i>Filicollis</i>	385
Fischthal, Jacob H. <i>Triganodistomum hypentelii</i> N. SP. (TREMATODA: LIS-SORCHIIDAE) FROM THE HOG SUCKER, <i>Hypentelium nigricans</i> (LE SUEUR)	389
Philip, Cornelius B. MECHANICAL TRANSMISSION OF RABBIT FIBROMA (SHOPE) BY CERTAIN HAEMATOPHAGOUS BUGS	395
Sprague, Victor and Juanita Ramsey. FURTHER OBSERVATIONS ON <i>Plistophora kudoii</i> , A MICROSPORIDIAN OF THE COCKROACH	399
Cable, R. M. and A. V. Hunninen. STUDIES ON THE LIFE HISTORY OF <i>Siphodera vinaleddwardsii</i> (LINTON) (TREMATODA: CRYPTOOGONIMIDAE)	407
RESEARCH NOTES.	
Erickson, Arnold B. and P. R. Highby. PARASITES OF THE WOODLAND CARIBOU	423
Rothschild, Miriam. A NOTE ON IMMUNITY REACTION IN THE BLACK-HEADED GULL (<i>Larus ridibundus</i> L.) INFECTED WITH <i>Maritrema oöcysta</i> LEBOUR, 1907	423
Hauschka, T. S. and M. I. Pennypacker. <i>Adelina deronis</i> N. SP., A NEW COCCIDIAN PARASITE OF THE AQUATIC OLIGOCHAETE, <i>Dero limosa</i>	424
Levine, P. P. EXCYSTATION OF COCCIDIAL OÖCYSTS OF THE CHICKEN	426
Schneider, Morris D. A NEW THERMOSTABLE MEDIUM FOR THE PROLONGED BACTERIA-FREE CULTIVATION OF <i>Trichomonas foetus</i>	428
Fuller, H. S. and Q. M. Geiman. SOUTH AMERICAN CUTANEOUS LEISHMANIASIS IN EXPERIMENTAL ANIMALS	429

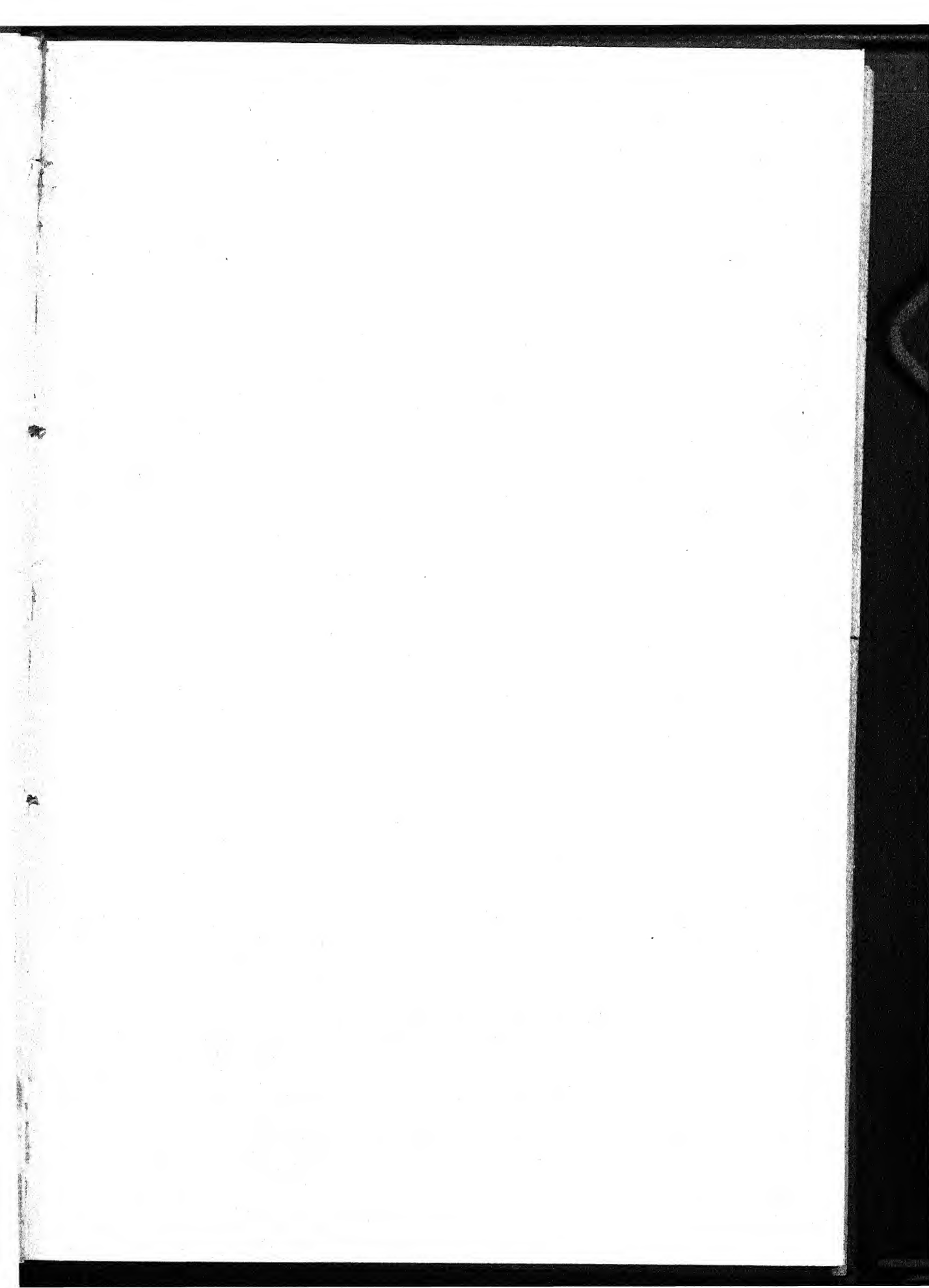
Forbes, William C. HELMINTHS FROM THE NORWAY RAT IN NORTH-EASTERN OHIO	431
Macy, Ralph W. THE LIFE CYCLE OF THE TREMATODE <i>Echinostomum cal-lawayensis</i> BARKER	431
AMERICAN SOCIETY OF PARASITOLOGISTS.	
PRELIMINARY ANNOUNCEMENT, 18TH ANNUAL MEETING, NEW YORK CITY, DECEMBER 28-30, 1942	432

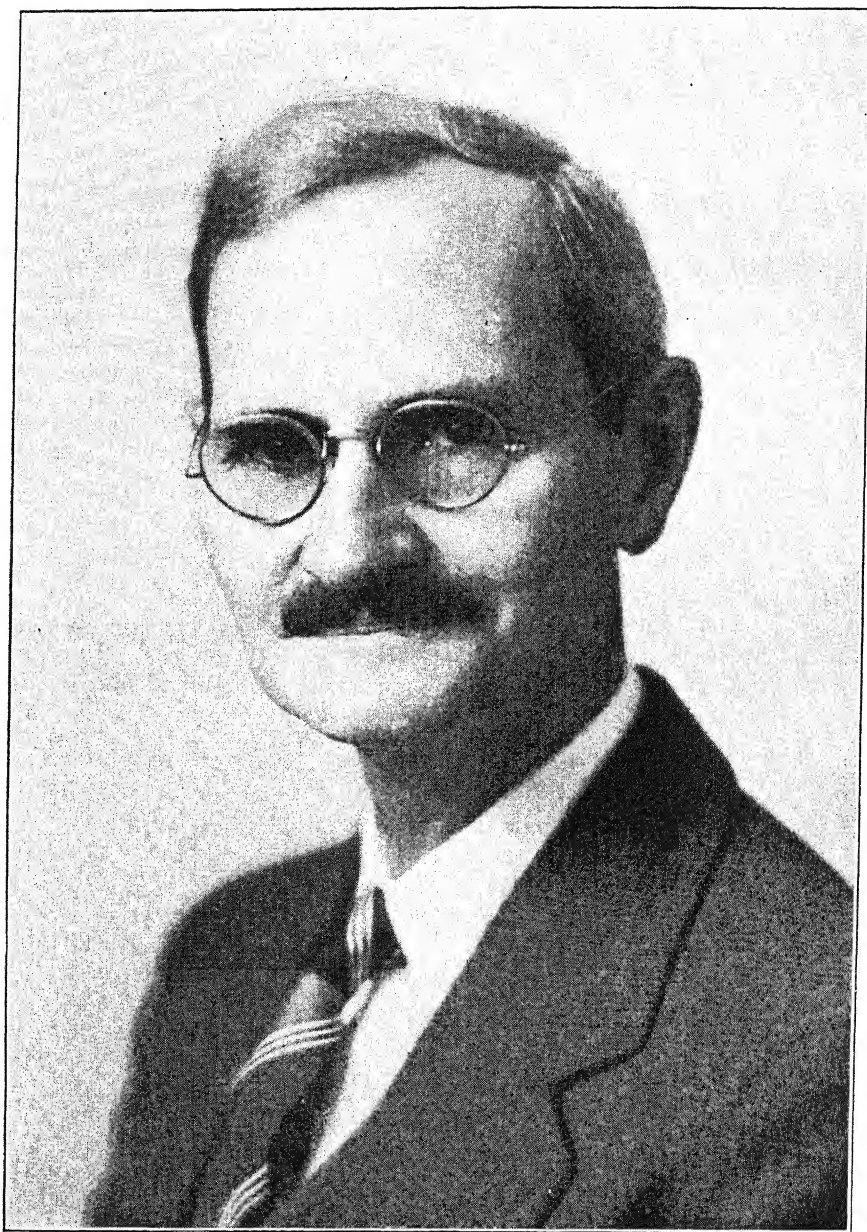
DECEMBER, 1942, NUMBER 6.

Dinaburg, A. G. THE EFFICIENCY OF THE BAERMANN APPARATUS IN THE RECOVERY OF LARVAE OF <i>Haemonchus contortus</i>	433
Rodaniche, Enid C. and Joseph B. Kirsner. THE EFFECT OF SULFONAMIDE COMPOUNDS ON THE GROWTH OF <i>Endamoeba histolytica</i> IN CULTURE	441
Goble, Frans C. and Arthur H. Cook. NOTES ON NEMATODES FROM THE LUNGS AND FRONTAL SINUSES OF NEW YORK FUR-BEARERS	451
Trager, William. A STRAIN OF THE MOSQUITO <i>Aedes aegypti</i> SELECTED FOR SUSCEPTIBILITY TO THE AVIAN MALARIA PARASITE <i>Plasmodium lophurae</i> ..	457
Williams, Charles O. OBSERVATIONS ON THE LIFE HISTORY AND TAXONOMIC RELATIONSHIPS OF THE TREMATODE <i>Aspidogaster conchicola</i>	467
Levin, A. J. and T. C. Evans. THE USE OF ROENTGEN RADIATION IN LOCATING AN ORIGIN OF HOST RESISTANCE TO <i>Trichinella spiralis</i> INFECTIONS ...	477
Ewing, H. E. REMARKS ON THE TAXONOMY OF SOME AMERICAN CHIGGERS (TROMBICULINAE), INCLUDING THE DESCRIPTIONS OF NEW GENERA AND SPECIES	485
RESEARCH NOTES.	
Harkema, Reinard. THE MOURNING DOVE, A NEW HOST OF THE ANO-PLOCEPHALID TAPEWORM, <i>Aporina delafondi</i> (RAILLIET)	495
Moore, Donald V. AN IMPROVED TECHNIQUE FOR THE STUDY OF THE ACANTHOR STAGE IN CERTAIN ACANTHOCEPHALAN LIFE HISTORIES ...	495
COMMUNICATION: NOTICE TO MEMBERS, SUBSCRIBERS, AND AUTHORS	496
INDEX FOR VOLUME 28, NUMBERS 1-6	497
(For December Supplement consult its Author Index and Program.)	
AMERICAN SOCIETY OF PARASITOLOGISTS.	
ANNOUNCEMENT OF POSTPONEMENT OF NEW YORK MEETING	506

DECEMBER SUPPLEMENT, 1942.

AMERICAN SOCIETY OF PARASITOLOGISTS.	
PROGRAM, 18TH ANNUAL MEETING, NEW YORK CITY, DEC. 28-30, 1942	1
AUTHOR INDEX	7
PROGRAM, SYMPOSIUM ON TROPICAL DISEASES, NEW YORK CITY, DEC. 29, 1942	8
ABSTRACTS	9
OFFICERS	31
IN MEMORIAM	34
LIST OF MEMBERS	35





J. E. Ackert

The Journal of Parasitology

Volume 28

FEBRUARY, 1942

Number 1

NATURAL RESISTANCE TO HELMINTHIC INFECTIONS^{1,2}

J. E. ACKERT

Zoology Department, Kansas State College

INTRODUCTION

Following the custom of recent years of choosing for this occasion a topic from one's investigational field, I have selected the subject natural resistance to helminthic infections. To follow the ramifications of this subject to their entirety would require more space and time than are available. It will be my purpose to review and analyze literature on such factors in natural resistance as diet, genetic constitution and age and to present some new data on an inhibitory nematode growth factor in duodenal mucus of older animals.

During the last decade there has been much confusion in the use of the terms resistance and immunity. In his excellent review, Taliaferro in 1929, used immunological terms to include essentially all phases of resistance. Natural resistance was considered by him an age immunity. About the same time Stoll (1929) discovered an immunity to helminthic infections to which he gave the name "self cure." Acquired resistance was used by Schwartz, Alicata and Lucker (1931), Chandler (1932a), Taylor (1934), Graham (1934), Lapage (1937) and others to designate an immunity due to previous helminthic infections.

In a review of literature from 1929 to 1938, Culbertson used immunity to cover both immunological and resistance phases whereas, in his 1941 review natural resistance and age resistance are considered apart from acquired immunity. Finally, a standard text book of bacteriology in its 1941 edition uses resistance and immunity synonymously.

In the present discussion the term natural resistance will be used to designate a host's resistance, at any age, to its helminths as developed in the absence of helminthic antigens. Natural immunity is interpreted as representing a natural incompatibility between the host and an invading

¹ Address of the Retiring President, American Society of Parasitologists, December 30, 1941, Dallas, Texas.

² Contribution No. 232 from the Department of Zoology, Agricultural Experiment Station, Kansas State College of Agriculture and Applied Science, Manhattan, Kansas.

helminth, while immunity will be used to represent the host condition that is due to helminthic antigens.

DIETARY FACTORS IN RESISTANCE TO HELMINTHS

Vitamins

In the fall of 1921 an extensive experiment was begun by myself and C. A. Herrick upon effects of the large roundworm of fowls (*Ascaridia galli*)³ upon its host. This experiment, we have been advised and so far as we have been able to ascertain, initiated experimental helminthology in this country. It yielded an interesting by-product, namely, an experimental demonstration of marked resistance of the hosts to the development of the nematodes (Ackert and Herrick, 1928).

While this study was in progress, a survey of the intestinal helminths of 1,000 chickens in the vicinity of Manhattan, Kansas, showed that nearly half of them were infected with *Ascaridia galli* (Ackert, 1927). Inquiry into the methods of rearing showed that the fowls in the garden season were usually confined to a pen in which no green feed was available. The principal feed of kaffir or corn was inadequate and low in vitamins B and A. The years, 1924 to 1927, which were devoted to studies of the resistance of chickens to *Ascaridia* as affected by diets deficient in vitamins B and A (Zimmerman, Vincent and Ackert, 1926; Ackert, Fisher and Zimmerman, 1927) gave experimental evidence, apparently for the first time in this country, that natural resistance of animals to helminthic infection may be lowered by nutritional deficiencies. In this period, an independent study of this nature was made in the Orient by Hiraishi (1926) working with swine and ascarids.

The general plan of our experiments was to work with growing chickens of the same ages obtained from a commercial hatchery and raised under carefully controlled conditions. Such chickens were divided into the various groups, some of which were subjected to the deficient diets and others kept as controls. The same numbers, 50 to 500, infective, *Ascaridia* eggs were fed to all chickens under comparison. At the close of the experiment, the fowls were sacrificed and the worms recovered by the flushing system of Ackert and Nolf (1929). Both the numbers and the lengths of the *Ascaridia* were used as criteria of host resistance.

In our experiments the fowls on the vitamin A deficient diets⁴ had markedly more and longer worms than did the control chickens (Ackert et al, 1931). Hiraishi (1926), working concurrently in Japan, fed pigs a diet deficient in vitamin A and lowered their resistance sufficiently to

³ This nematode in the last twenty years, has been variously known as *Ascaridia perspicillum*, *A. lineata* and *A. galli*.

⁴ White corn meal, vitamin A-free casein, yeast, vegetable fat, bone ash and salt mixture, and irradiation of fowls.

parasitize them with the human *Ascaris*. Payne, Ackert and Hartman (1925) had shown that the human *Ascaris* larvae would pass through the somatic phase of the life cycle in swine but not the intestinal phase in swine kept on an adequate diet. Clapham (1933) working on the hatching and survival of the horse ascarid, *Parascaris equorum*, in rats found vitamin A to be an important factor in resistance. Clapham's (1933) results on *Heterakis gallinae*, the cecal worm of chickens, indicated in two of three tables of data that vitamin A is a factor in the resistance of chickens to this nematode, although she did not so interpret her results. Judging from egg counts of the swine *Ascaris*, Clapham (1934) failed to obtain evidence that vitamin A deficiency increased the productivity of the ascarids. However, Wright (1935), who subjected dogs infected with ascarids to diets partially or totally deficient in vitamin A for periods of 15 to 106 days, found that the dogs on the deficient diets harbored about five times as many ascarids as did the controls on an adequate diet and exposed to the same degree of worm infection.

More critical studies on the effect of vitamin A deficiency on the resistance of rats to infection with *Trichinella spiralis* were made in 1934 by McCoy who tested the efficacy of the deficient diet by making determinations of the amount of vitamin A stored in the livers of the experimental rats. His findings supported those of previous workers with other nematodes and showed that vitamin A is an important factor in the reaction of the host to superinfections of *Trichinella spiralis*. Lawler's recent (1941) studies with the rat nematode *Strongyloides ratti* likewise showed that the absence of vitamin A lowered the rats' resistance to a primary infection and weakened the immunity to reinfection with the nematodes.

These and the other results here reviewed point strongly to the conclusion that a diet markedly deficient in vitamin A will lower the resistance of the host to its nematode parasites. The reason for this effect has not been completely elucidated. It is known that lack of vitamin A in the system affects the normal functioning of epithelial membranes of parts of the respiratory, digestive and urino-genital tracts and also those of the eyes and tear ducts. Whether the lining of the small intestine which is the habitat of most of the adult nematodes studied, is affected will require further study as will also effects on organs and tissues of the body.

As mentioned previously, early experiments on vitamin B deficiency gave evidence of lowered host resistance to the fowl *Ascaridia* (Zimmerman, Vincent and Ackert, 1926). After the multiple nature of vitamin B was established, Ackert and Nolf (1931) obtained rather striking results on numbers of worms. The lack of vitamin B which caused partial paralysis of the muscles of the digestive tract resulted in the reten-

tion of a high percentage of the worms, whereas the control chickens eliminated most of theirs.

Foster and Cort (1931) by using a diet⁵ deficient in vitamins A and B and minerals lowered the resistance of dogs previously infected with the hookworm, *Ancylostoma caninum*. After the productivity of the parasites had increased markedly in the dogs on the deficient diet, the hosts were returned to an adequate diet which increased the resistance and caused an extreme inhibition of the egg production of the worms. Results from other experiments by these authors (1935) indicated that as the dogs on the deficient diet became more and more emaciated, the development and productivity of the worm parasites increased. The results of this extensive series of experiments furnish further evidence that vitamins A and B are important factors in host resistance to helminthic infections.

Among the early difficulties in the rearing of chickens in confinement was the development of a form of rickets which is caused by insufficient vitamin D. Tests on this vitamin by Ackert and Spindler in 1929 indicated that vitamin D protects the hosts against the effects of parasitism rather than by inhibiting the development of worms.

On the subject of vitamins, by the way, a struggling student wrote, "Lack of vitamin A is not as bad as lack of vitamin B which in turn will not have so many bad effects as will the lack of vitamin C and so on down the alphabet!"

Studying diet as a factor Chandler in 1932 placed rats on a diet wholly of vegetables and fruit and inferred that diet played no part in the acquisition or retention of worms so long as the host animals were not injured by the diet. But when emaciation began to develop the rats acquired more worms from a given inoculation than did the controls on more favorable rations. As this diet was deficient in vitamin B these results give additional evidence of the importance of this vitamin in natural resistance to helminths.

Other Dietary Supplements

The bare pens and cereal rations of many of the 1,000 chickens examined at Manhattan, Kansas, in the early twenties (Ackert, 1927) indicated that the fowls were being kept much of the year on rations that were almost wholly of plant origin. Preliminary tests by Ackert indicated that the inclusion of skim milk as a supplement to the diet produced chickens that were unusually resistant to the *Ascaridia*.

The studies continued by Ackert and Beach (1933) afforded evidence that a basal, cereal ration adequate for vitamins and minerals when supplemented by meat meal and by skim milk produced the most resistant

⁵ Corn starch, dried ground peas, mazola oil and sodium chloride.

fowls. The cereal ration plus meat meal was second best, whereas, the cereal ration supplemented by peanut meal (a wholly plant ration), produced the least resistance in the chickens to the *Ascaridia*. These results, which were attributed to the wider range of the amino acids made available from the diets of the first two groups, and to the restricted range of amino acids in the plant diet of the chickens of the third group throw light upon the results of Chandler (1932) in which markedly more *Nippostrongylus muris* were retained in rats whose diet was wholly of plant origin (vegetables and fruit); and also upon the results of Foster and Cort (1931) whose deficient diet for dogs lacked both animal tissues and milk.

That the resistance of hosts to tapeworms is affected by diet was shown by Shorb in 1933 working with rats and the tapeworm *Hymenolepis fraterna*. A ration of white bread and water which was deficient in vitamins, minerals, proteins, and fats lowered the resistance of the rats to the tapeworms.

From the foregoing considerations, it might appear that any deficient diet that lowers the resistance of the host, will permit the helminths to increase their development and productivity. However, such was not the case with the intestinal nematode, *Ascaridia galli* as judged by its inability to grow in a host that was on a very deficient diet. In a study on the nature of the food of *Ascaridia* (*A. galli*) Ackert and Whitlock (1935) by giving chickens intra-muscular injections of a nutrient solution lacking vitamins A and B and animal proteins, lowered the resistance of the chickens without enabling the worms to thrive in the bodies of the hosts. Although many of the injected chickens died of starvation and had their resistance completely broken, fewer and shorter worms remained in them than in the controls which were kept on the regular diet (Ackert, Whitlock and Freeman, 1940).

These results indicate that there may be a critical point in the broken resistance of a host beyond which the environment becomes unfavorable for the helminth. In the present case it might be argued that the absence of nutriment in the lumen of the chicken intestine caused partial starvation of the worms, but this period of parasitism included the seven days during which the anterior ends of the worms were deeply imbedded in the mucosa where the worms doubtless fed on host substance. As Bahrs (1931) found that planaria would not grow when fed on the intestinal epithelium of starved rabbits, but would grow when fed on that of normally fed rabbits, it is possible that the *Ascaridia* in the injected chickens were starved both from the lack of nutrient in the intestinal lumen and from the lack of food in the intestinal mucosa. At any rate, the broken resistance of the hosts did not result in increased development and productivity of their helminth parasites.

In this connection Zaiman (1940), who studied the effect of vitamin E deficiency on *Trichinella spiralis* infections in rats, found that this deficiency which may cause muscular dystrophy was less favorable for the trichinellae.

Bordering on the field of resistance as affected by diet are such studies as those of Clapham (1934a), Porter (1935b) and Otto and Landsberg (1940) on minerals, and of Reid (1940) on starvation. Clapham found that a diet markedly deficient in minerals (especially in calcium and phosphorus) favored the development of the fowl cecal worm *Heterakis gallinae*. Porter found that a diet markedly deficient in iron lowered the natural resistance of rats to the nematode *Nippostrongylus muris*. Otto and Landsberg determined that in dog hookworm disease iron therapy accompanying a deficient diet failed to affect the development or persistence of the worms, but that iron therapy may well supplement general dietary improvements. Reid found that host starvation of but 20 hours caused the removal from chickens of the complete strobila of the fowl tapeworm, *Railletina cesticillus*.

The literature here reviewed, while not permitting a blanket statement that deficient host diets react to the benefit of the parasite, nevertheless, indicates that in general the natural resistance is lowered to helminthic infections both in the somatic and intestinal phases, when omnivorous hosts are maintained on diets deficient in vitamins A, B (complex), or D; or on rations with highly restricted sources of proteins.

GENETIC CONSTITUTION AS A FACTOR IN RESISTANCE

Epidemiological Evidence of Group Differences in Helminthiasis

That natural resistance to helminthic infections may be due in part to the genetic constitution of the host is indicated by both epidemiological data and experimental results. Miller (1908) observed that the pure-bred Zebu cattle in Trinidad resisted the ravages of strongyles better than did other breeds of cattle; he also found that sheep succumbed to the attacks of the helminths more easily than did the cattle.

Race differences in resistance to hookworms were found by Smillie and Augustine (1925), who examined nearly 2,000 rural school children. The white children had both more hookworms and a higher percentage of infection than did the negro children of the same ages living in the same communities. The authors attributed the lighter negro infections to the much greater thickness of negro epidermis.

Further evidence of racial differences in resistance to hookworms is available from the observations of Keller, Leathers and Knox (1937) made in North Carolina. Examinations of several thousand white and colored persons showed that 12.3 per cent of white persons were positive for hookworm eggs as compared with 4 per cent for the negroes of

comparable ages. These observations and others made by Keller and his associates in southern United States indicate that the resistance of negroes from five to 19 years of age to hookworm infection is markedly higher than that of whites living in the same communities.

Race differences in *Ascaris* infections were noted by Keller, Leathers and Bishop (1932) who found in a Tennessee survey that persons of the white race had heavier *Ascaris* infections than did the negroes. However, in west Tennessee, the reverse proved true. Two years later Otto and Cort (1934) reporting for the age group 5–14 years in rural areas of four southern states found *Ascaris* more prevalent and of greater intensity in the negro youths than in the whites. These authors, however, doubted that the negro race is more susceptible to *Ascaris* infection than the white race. They felt that among the negroes of those areas there was a greater prevalence of habits that were favorable for *Ascaris* infections.

Observations on differences in racial resistance to tapeworms were made by Otto (1936) who found that the whites in the southern states (five to 14 years of age) had somewhat more tapeworms (*Hymenolepis nana*) than did the negroes of the same ages. The percentages of infection were likewise constantly higher in the whites than in the negroes.

Race differences to pinworms (*Enterobius vermicularis*) were found by Cram (1940) in a study of children in nursery schools and summer camps where 33 per cent of the white children showed worm infections as compared with 19.8 per cent of the negro children. The examinations of these children together with others totaling 3,371 showed that 41.9 per cent of the whites were infected as compared with 15.5 per cent of the negroes. These results which include samplings from different parts of the country furnish further epidemiological evidence that racial or genetic constitution may be a factor in natural resistance to helminthic infections.

The epidemiological evidence of race differences in resistance of people to helminths as here reviewed seems to indicate that negroes in the southern states have greater natural protection against helminths than do the whites. This was especially true of hookworms and pinworms, but included some evidence also on *Ascaris* and the dwarf tapeworm (*Hymenolepis nana*). It is difficult to conceive of the negroes living on a higher plane either from the standpoint of nutrition or of sanitation. In the case of pinworms harbored near the nation's capital it is possible that the white nursery school children became more readily exposed to infections from the parasite eggs on the hands in games like ring-around-the-rosy than were the negro children of that region. The incidence of the various helminths probably is due to several factors, but no discussion of this subject would be complete without raising the ques-

tion of the negroes' longer association with the helminths than whites'. On the host-tolerance hypothesis it would be expected that the longer association of the negro race with the worms would result in better host-parasite adaptation and a consequent lowering of resistance to the nematode. On this basis, however, the negro infections would be larger instead of smaller. On the racial immunity hypothesis the longer association of the negroes with the worms would afford greater opportunity for the development of a grade of immunity. Whether this would become inheritable by mutations or by other means is problematical.

A desperate student who had neglected his studies once wrote in an examination, "Heredity is harmful, and should be prevented."

Experimental Evidence of Breed and Strain Differences in Resistance

The first experimental evidence of breed differences in resistance to helminthic infections appears to be from the work of Ackert, Eisenbrandt, Glading and Wilmoth in 1933 who found significant differences in the natural resistance of breeds of chickens to the large roundworm *Ascaridia galli*. Such heavy breeds and varieties as Rhode Island Reds, White Plymouth Rocks and Barred Plymouth Rocks had significantly fewer and smaller *Ascaridia* than did the lighter White Leghorn and White Minorca breeds (Ackert et al, 1935).

What appears to be the first experimental evidence of genetic differences in strains of hosts to helminthic infections was furnished by Curtis, Dunning and Bullock (1933) working with the larval tapeworm *Cysticercus fasciolaris* which sometimes caused liver tumors. The study demonstrated that some strains of rats reacted more often to the production of tumors than did others.

A strain of heavy White Minorca chickens in our studies proved to be more resistant to the *Ascaridia* than a lighter strain of the same breed with different genetic constitution (Ackert and Wilmoth, 1934). Bronze turkeys were found by Ackert and Eisenbrandt (1935) to be more resistant to *Ascaridia* of chickens than were White Leghorn fowls of the same age. Additional evidence of genetic strains within a breed was obtained by Ackert, Pratt and Freeman (1936) who, by selecting the more resistant cockerels and pullets from one flock of chickens, developed a strain of White Leghorns that was more resistant to the nematodes than were a cockerel and six hens taken at random from another flock of the same breed. Father-daughter and brother-sister matings were made in each group and all parasitized alike. The results of such tests carried on for three generations indicated that it would be possible to establish by selection a strain of chickens that is quite resistant to the *Ascaridia* as well as one that is distinctly susceptible to this nematode.

Indications of differences in breed resistance of sheep to helminthic

infections were obtained by Cameron (1935) who found that Cheviot sheep on an overcrowded pasture were more tolerant of the effects and less heavily parasitized with nematodes than were the lowland breeds such as the Border Leicester. The Shetlands and Scottish Blackface sheep were about intermediate between the other two groups. Differences in breed resistance of sheep to the nematode *Ostertagia circumcincta* were found by Stewart, Miller and Douglas in 1937 when Romney sheep were found to be significantly more resistant to the *Ostertagia* than were the Shropshires, Southdowns or Hampshires.

These data on the different degrees of sheep breed resistance to the stomach worm *Ostertagia circumcincta* were studied by a geneticist, Gregory (1937), with a view of prescribing a method of establishing within breeds, lines that are genetically resistant to the worms. The accumulating evidence pointed toward the same hereditary factors for resistance to *O. circumcincta* as to another sheep stomach worm *Haemonchus contortus* which is of paramount importance in much of the United States. Gregory's study of the data led him to think that resistant lines of sheep to stomach worms could be established without sacrificing type, conformation, quality of carcass or desirable wool characteristics.

As a step in the study of producing a resistant line of sheep to stomach worms, Gregory, Miller and Stewart (1940) tested offspring of two Hampshire rams by making fecal egg counts of trichostrongyles except *Nematodirus*. While variables of season of the year and age of the ewes were encountered, unquestioned evidence was obtained for genetic constitution as a factor. The two sires studied differed in genetic constitution for resistance and susceptibility to stomach worm infections, the offspring of one having significantly fewer worm eggs than did those of the other ram. From the results obtained, the authors felt that genetic selection should effectively change the degree of resistance or susceptibility to strongyles in populations of sheep.

The few experimental findings in the field of genetic constitution as a factor in natural resistance to helminthic infections are quite promising especially among animals that do not suffer deterioration from inbreeding as usually is the case with chickens. It is hoped that in the next decade this field may be much more fully investigated.

AGE RESISTANCE TO HELMINTHIC INFECTIONS

Experimental Evidence

The first experimental evidence of age as a factor in natural resistance to helminthic infections appears to be that of Looss (1911) who in feeding hookworm larvae to dogs observed that in young animals some of the larvae were able to reach maturity, whereas, previous investiga-

tors had failed to obtain such results in adult dogs. In 1920, Ransom and Foster working with *Ascaris* of pigs confirmed Looss's finding. The next year Ransom (1921) detected definite age resistance of chickens to the gapeworm *Syngamus trachea*.

It was at this time that Ackert and Herrick obtained experimental evidence of increased resistance with age, to the fowl ascarid of chickens (reported in 1928). A critical study of age resistance of chickens to this nematode was begun by Herrick in our laboratory in 1923-24 and concluded by him the next year in Dr. W. W. Cort's laboratory at Johns Hopkins University (Herrick, 1926). Herrick found a gradual increase in the resistance of chickens as they became older; the resistance increased with age up to 103 days after which no further increase was found in the fowls. In a 10-day period, the worms grew more than ten times as much in five-day chicks as in 103-day old fowls.

Herrick (1928) also demonstrated age resistance of dogs to hookworms. His findings were confirmed by Scott (1928) and Sarles (1929a) who also found age resistance of cats to hookworms.

That older rats develop an increased resistance against the nematode *Nippostrongylus muris* was shown by Africa (1931). Another rat parasite, the cestode *Hymenolepis fraterna* which also infects the mouse, was shown by Shorb (1933) to encounter greater resistance in older animals. Shorb found in both nursing rats and mice an initial resistance which was lost a short time after weaning.

In an extensive study of the rat nematode *Heterakis spumosa*, Winfield (1933) found an increased resistance with age of rats to this nematode. Twenty days in the life of young rats was sufficient for them to develop significantly greater resistance to the worms. Chickens develop resistance more rapidly, as shown by Ackert, Porter and Beach (1935) who found that from five- to eight-day periods in chickens about two months old may result in significant increases in resistance to growth of *Ascaridia*. Using approximately thirty-day infecting intervals Roberts (1937) obtained similar results with fowls and *Ascaridia*.

Age resistance was found by Kauzal (1934) to develop in sheep to lung worms (*Dictyocaulus filaria*) and by Roberts (1939) in cattle to gastro-intestinal helminths. Increased resistance with age was reported in 1934 by Ameel in snails to larval flukes and in 1936 by Davis in recently metamorphosed frogs to cercariae. Further evidence of host age resistance to flukes was presented by Cable (1937) experimenting with metacercariae (*Parorchis acanthus*) and gulls (*Larus argentatus*).

That tapeworms encounter more resistance in older hosts than in younger ones was shown by Ackert and Reid in 1937 working with chickens and the fowl tapeworm *Raillietina cesticillus*. The liver nematode *Capillaria hepatica* in rats and mice was shown by Luttermoser

(1938) to meet greater resistance in the older hosts, the rats showing more resistance than the mice. Rats also manifested an age resistance to *Trichinella spiralis* as shown by Nolf and Zaiman (1941). In the tests the nursing rats, however, had somewhat fewer trichinellae than the recently weaned ones.

This review of experimental work indicates that the phenomenon of increased resistance with age may occur in mammalian, avian, amphibian and molluscan hosts to trematode, cestode or to nematode helminths.

A phenomenon that may or may not be associated with age was the observation by Shorb (1933) that nursing rats gave evidence of a strong natural resistance to tapeworms and a similar finding by Nolf and Zaiman (1941) of stronger resistance in nursing rats against trichinellae than in weaned ones. It is evident from these findings that nursing was unfavorable for the development of the worms. Whether this was due to a vermifugal action of the mother's milk, to a highly restricted protein diet, or to some other factor is not known.

Epidemiological Evidence of Age Resistance

An erroneous idea crept into the literature when Smillie's (1922) epidemiological study of hookworm disease in Brazil indicated no age resistance of man to these nematodes. General works have carried the statement, and it has appeared in a review as recently as 1938. A decade passed before observations by Keller, Leathers and Ricks (1934) indicated that after 15–19 years of age, people do develop a resistance to hookworms. They found that the worm incidence fell off rather sharply after the age of 24 years. A series of studies by Keller, Leathers and their associates (Keller et al, 1937; Leathers et al, 1939) on hookworm infections in four southern states gave essentially similar results on age resistance of people to hookworm, namely a gradual increase in incidence up to 15 to 19 years of age after which there was a lower percentage and a lower intensity of hookworm infection.

In the case of the human *Ascaris*, the evidence of age resistance was positive from the first observations. The peak of infection percentages in the findings of Cort and Otto and their associates (Cort, Otto and Spindler, 1930; Otto and Cort, 1934) in the southern states occurred in the age group of seven to 14 years. The group 15 to 19 years old manifested much lower percentages of infections with these helminths. Increased resistance was found by Cram and Reardon (1939) to the pinworm *Enterobius vermicularis* in examination of 2,097 persons residing in the vicinity of Washington, D. C. Youths from six to 18 years of age had a pinworm incidence of 51 per cent as compared with 22 per cent for adults.

Clinical manifestations of schistosomiasis were found by Fisher

(1934) to be especially common in Belgian-Congo children under ten years of age but never in persons over 30 years old. The epidemiological evidence here reviewed indicates that host resistance increases with age to many species of nematodes and to some cestodes and trematodes.

ATTEMPTS TO EXPLAIN AGE RESISTANCE

Although three decades have passed since Looss found that infection experiments with parasites were more likely to succeed with young hosts, we are still trying to solve the riddle of age resistance to parasitism. Sandground (1929) in a stimulating review of host-helminth relation to age resistance and acquired immunity seriously questioned the existence of host age resistance to a helminth that is adjusted to its host. He stated, "It is now becoming evident that age resistance is not of general occurrence in helminth infections, nor is the real extent of its occurrence known." He sought to explain the occurrence of age resistance to helminths on the basis of an abnormal host hypothesis. Of the findings of Herrick (1928) and Scott (1928) on age resistance of dogs and cats to *A. caninum*, Sandground held that compared with *A. duodenale* of man, *A. caninum* is poorly adapted to both the dog and the cat. The large roundworm of fowls, *A. galli*, Sandground considered as essentially the parasite of the young chicken. He pointed to the high correlation between the exhibition of age resistance and a greater or lesser condition of abnormality in the species relation of the parasite and the host, and felt that the factors involved in age resistance could be explained by an extension of those factors required for the understanding of natural immunity.

In the same year Sarles (1929b) attributed age resistance of dogs to hookworm larvae to the action of the body defense cells since larvae penetrating the host skin caused marked local cellular reaction in old dogs but not in young ones. After Africa (1931) found evidence of age resistance of rats to *Nippostrongylus muris*, Chandler (1932b) who found somewhat less striking evidence of age resistance of rats to this parasite sought to account for the discrepancy between his work and Africa's on a host-parasite adaptation hypothesis. His larvae had been through several generations in laboratory animals whereas those of Africa had been only recently isolated. However, Graham and Porter (1934) were unable to find evidence of an adaptation of an old laboratory strain when compared with a recently isolated strain of *N. muris*.

Some support for the abnormal host hypothesis of age resistance to helminths was furnished by Porter (1935a) who studied the behavior of *Nippostrongylus muris* in rats and mice. The larvae which were obtained from a rat strain of the nematode developed more readily in rats than in mice. Porter found evidence of an age resistance in both

rats and mice to *N. muris* and observed that this increased resistance developed earlier and to a more marked degree in mice than in rats. Porter thus leaned toward the abnormal host hypothesis for the marked age resistance of the mice to the parasite but did not give reasons for the increased resistance of the older rats which presumably were the normal hosts.

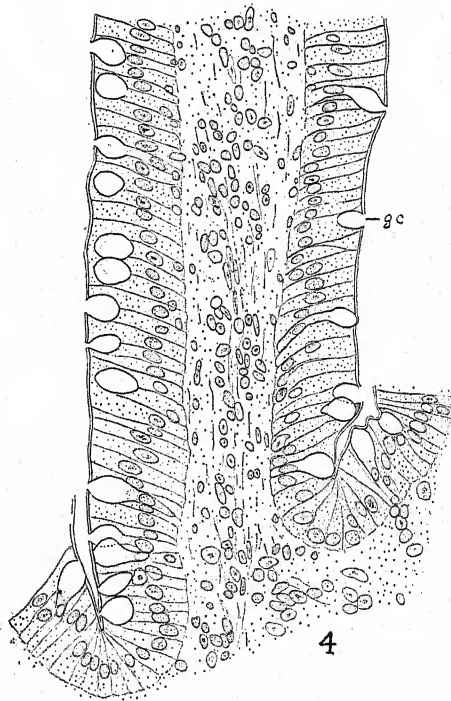
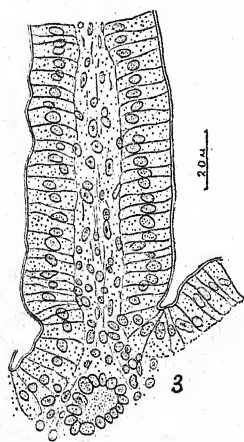
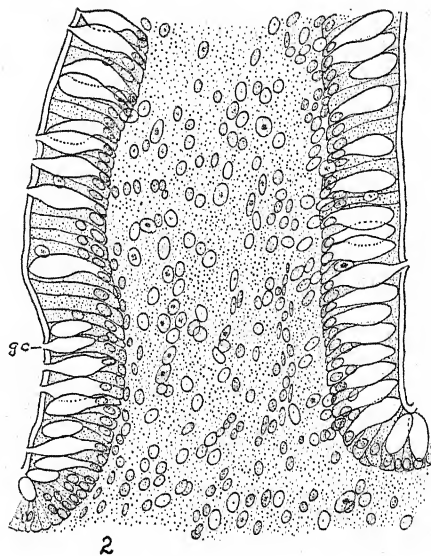
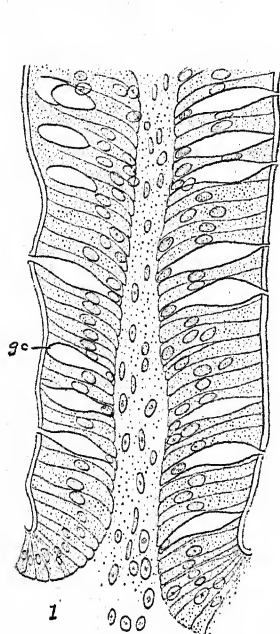
In 1935, Ackert, Porter and Beach sought to explain age resistance of chickens to *Ascaridia* on the hypothesis of a growth-inhibiting substance since Carrel and Ebeling (1921) had shown the presence of an active growth-inhibiting substance in the blood serum of chickens, and Ackert and Porter had found that bleeding lowered the resistance of the chickens to the worms (Ackert, 1925; Porter and Ackert, 1933). It was then felt that the resistance mechanism might be in the form of a fluid that passes from the intestinal wall into the lumen, or a deposit in the mucosa, or that the cellular mucosa which the young worms puncture might become tougher in the older chickens.

Foster (1936) who produced anemia in dogs by keeping them on a diet deficient in iron found a parallel between resistance and the hemoglobin level of the host. His results led him to believe that age resistance of the dogs against the hookworm *A. caninum* may be related in part to the natural age curve of the hemoglobin level of the host.

The increased resistance of older amphibia to the penetration of cercariae of *Diplostomum flexicaudum* was attributed by Davis (1936) to the greater thickness of the skin of recently metamorphosed frogs than of large tadpoles. Davis felt that in the frogs the integumentary barrier was either too thick and horny for the cercariae to pierce, or that the mucous and poison gland secretions of the skin killed, or inactivated them.

Duodenal Mucus and Age Resistance

In a search for possible structural differences in the habitat of *Ascaridia* in younger than in older chickens, Ackert and Edgar (1938) made histological studies of portions of the duodena of such chickens. There were some differences in thickness of muscular layers, but the most striking difference was the increasing numbers of goblet cells in the chickens as they grew older (Figs. 1, 2). Studies made on chickens from two to 320 days of age showed gradual increases in the number of goblet cells per area of duodenal epithelium up to the age of 124 days. Increases corresponded closely to the manifestations of age resistance as shown by Herrick (1926). It was felt that the secretions of the goblet cells might be a factor in the elimination of the worms from the host since it had been observed repeatedly on opening the duodenum that the mucus was much more abundant in older chickens than in the younger ones. This appeared to be the first record of increased numbers of goblet



cells in older animals and of their possible relations to age resistance to intestinal parasitism.

Similar histological studies made on the duodenal epithelium of a number of laboratory rats of different ages showed that older rats likewise have larger numbers of duodenal goblet cells per area than do younger ones (Figs. 3, 4), but the increase is more gradual. The results, however, showed that the phenomenon of increased numbers of duodenal goblet cells is characteristic of a mammal as well as a fowl. As shown previously rats develop age resistance to their intestinal nematodes, but the resistance does not develop as rapidly in the rat as it does in the chicken. Correspondingly, the numbers of duodenal goblet cells do not increase as rapidly in the rat as they do in the chicken (Ackert, Edgar and Frick, 1939).

An Inhibitory Growth Factor in Duodenal Mucus

To test secretions of the goblet cells for an inhibitory growth factor duodenal mucus removed quickly with a blunt spatula was autoclaved and used in the ratio of 3 cc to 65 cc of the nutrient medium, that Ackert, Todd and Tanner (1938) developed for in vitro culture of *Ascaridia*. The pH of the mucus solution was adjusted to that of the control solution (approximately 6.7, as found by Ackert, 1931, for the worms' habitat in the fowl intestine). Young nematodes (*A. galli*) of the same age were removed from their normal chicken hosts, washed in warm saline solution, photographed for measurement and placed in dishes containing one of three solutions. At the end of about three days all worms were re-measured. The results of the first experiment showed that in a non-nutrient isotonic salt solution the worms lost an average of 2.5 mm. In the nutrient solution (isotonic salt-dextrose) the worms grew 11.8 mm or an increase of 18.5 per cent, whereas the worms in the medium that contained the mucus grew only 2.2 mm or an increase of 4 per cent (Table 1). The results of this test thus indicated that the mucus contained a factor that was unfavorable to the growth of the young *A. galli*.

EXPLANATION OF FIGURES

The figures were drawn with the aid of a camera lucida. They are all of the same magnification.

Abbreviations: *gc*, goblet cell.

FIG. 1. Median section through basal portion of a duodenal villus from a 5-day chick.

FIG. 2. Median section through basal portion of a duodenal villus from a 124-day chicken.

FIG. 3. Median section through basal portion of a duodenal villus from a 7-day rat. Note the absence of goblet cells.

FIG. 4. Median section through basal portion of a duodenal villus from a 646-day rat.

(From Ackert, Edgar and Frick, 1939.)

TABLE 1.—Results of tests for an inhibitory nematode growth factor in duodenal mucus from chickens three months old (From Ackert, Edgar and Frick, 1939)

Culture media	Larvae in culture (days)	Gain in length average (mm)	Per cent gain
Experiment I			
*Isotonic salt-dextrose solution plus mucus	3	2.2	4.0
Isotonic salt-dextrose solution (control)	2.5	11.8	18.5
Isotonic salt solution (no nutrient)	2	2.5 (loss)	0.0
Experiment II			
Isotonic salt-dextrose solution plus mucus	3	8.5	12.8
Isotonic salt-dextrose solution (control)	3	27.0	36.9
Isotonic salt solution (no nutrient)	3	0.2 (loss)	0.0
Experiment III			
Isotonic salt-dextrose solution plus mucus	3	2.1	2.9
Isotonic salt-dextrose solution (control)	3	14.3	19.8
Isotonic salt solution (no nutrient)	4	11.0 (loss)	0.0

* The pH of the isotonic salt-dextrose solution was 6.1; that of the autoclaved mucus was 6.0.

The results from the second and third experiments were much like those of the first, thus indicating that the autoclaved mucus from the duodenal goblet cells of chickens three months old contained a factor which retarded the growth of the *Ascaridia* in vitro.

Tests were next made with autoclaved duodenal mucus from chickens about four months (125 days) old (Table 2). The young nematodes in the nutrient medium plus mucus for three to four days either grew but little or lost size in this medium the average for the four lots being a loss of length of 4.66 per cent. On the other hand young worms in the control cultures gained an average of 12.15 per cent.

TABLE 2.—Test for inhibitory nematode growth factor in duodenal mucus (autoclaved) from 125-day chickens

No.	Culture	Days in	Length in (mm)	Length out (mm)	Gain in length	Loss in length*	Per cent gain
9M1	Iso-salt + dex. + mucus	4.5	91.50	83.50	8.00
9M2	Iso-salt + dex. + mucus	3.0	61.50	64.00	2.50	...	4.06
9M3	Iso-salt + dex. + mucus	3.5	58.98	57.00	1.98
9M4	Iso-salt + dex. + mucus	4.5	97.98	87.50	10.48
						Av. loss	4.66
	(Control)						
9D1	Iso-salt + dex.	3.0	58.98	66.00	7.02	...	11.9
9D2	Iso-salt + dex.	4.5	88.98	100.00	11.02	...	12.4
						Av.	12.15

* Per cent determined, but not here recorded.

The next tests were made on mucus from chickens about two months (66 days) of age. The mucus was autoclaved as usual and introduced at the ratio of 3 cc to 65 cc of nutrient medium. The young worms in six such cultures from one to three days made in each case a substantial gain in length, an average of 30.58 per cent. The controls gained but little more, an average of 40.75 per cent (Table 3). These results indicated that while there was some retardation of the growth of the worms, the inhibiting factor in the two-month mucus was not as potent as in the mucus from the 125-day chickens.

TABLE 3.—*Test for inhibitory nematode growth factor in duodenal mucus (autoclaved) from 66-day chickens*

No.	Culture	Days in	Length in (mm)	Length out (mm)	Gain in length	Per cent gain
3M1	Iso-salt + dex. + mucus	3	48.75	67.00	18.25	37.4
3M2	Iso-salt + dex. + mucus	2	37.50	53.66	16.16	43.0
3M3	Iso-salt + dex. + mucus	3	52.50	75.00	22.50	42.8
3M4	Iso-salt + dex. + mucus	1	52.50	59.50	7.00	13.3
3M5	Iso-salt + dex. + mucus	2	30.83	38.50	7.67	24.8
3M6	Iso-salt + dex. + mucus	3	43.75	53.50	9.75	22.2
						Av. 30.58
(Control)						
3DC1	Iso-salt + dex.	2	35.00	55.50	20.50	58.5
3DC2	Iso-salt + dex.	1	48.75	60.00	11.25	23.0
						Av. 40.75

The question arose as to whether the substance responsible for the inhibitions of the growth of the nematodes was soluble. Accordingly, the duodenal mucus from chickens 171 days old after being autoclaved was ground with sterile sand and a 0.9 per cent saline extract prepared. This was used in three nutrient cultures in the place of mucus; also in four other cultures, washings from autoclaved mucus were added to the nutrient media; four nutrient control cultures were prepared. After being in these respective cultures for from six to nine days, it was found that in the nutrient plus mucus extract cultures one group had gained while two had lost in length giving an average loss of 7.2 per cent. In the nutrient plus mucus washings cultures one group gained while three lost in length giving an average loss of 1.79 per cent. The worms in the control cultures all increased in length, gaining an average of 9.25 per cent (Table 4). The results from this test indicated that the growth-retarding factor in the autoclaved mucus was soluble to some extent in 0.9 per cent saline. Subsequent tests made on the above washings indicated that the inhibitory factor was capable of passing through a Berkefeld filter. That the effect of the inhibitory growth factor did not produce permanent effects upon the worms was shown in a subsequent test in which the worms of cultures 11G1 and 11G3 (Table 4) on being

TABLE 4.—Test for inhibitory nematode growth factor in duodenal mucus (autoclaved) from 171-day chickens

No.	Culture	Days in	Length in (mm)	Length out (mm)	Gain in length	Loss in length*	Per cent gain
11G1	Iso-salt + dex. + ground mucus extract	8	66.30	59.50	6.80
11G3	Iso-salt + dex. + ground mucus extract	8	73.10	59.50	13.60
11G4	Iso-salt + dex. + ground mucus extract	6.5	57.80	63.50	5.70	9.85
						Av. loss	7.23
11W1	Iso-salt + dex. + mucus washings	9	76.50	89.25	12.75	16.60
11W2	Iso-salt + dex. + mucus washings	8	73.10	67.66	5.44
11W3	Iso-salt + dex. + mucus washings	9	64.60	62.50	2.10
11W4	Iso-salt + dex. + mucus washings	8	73.64	64.00	9.64
						Av. loss	1.79
	(Control)						
11D1	Iso-salt + dex.	6	66.30	77.00	10.70	16.14
11D2	Iso-salt + dex.	6	56.10	60.00	3.90	6.95
11D3	Iso-salt + dex.	6.5	68.00	75.00	7.00	10.29
11D4	Iso-salt + dex.	6	71.40	74.00	2.60	3.64
						Av.	9.25

* Per cent determined, but not here recorded.

placed in fresh nutrient media made an average gain of 32.7 per cent in six days.

In a series of tests young *A. galli* were cultured in media to which was added autoclaved duodenal mucus from chickens arranged in a graded series from susceptible to resistant hosts ranging in ages from 45 to 125 days (Table 5).

Worms subjected to 45-day mucus made as much gain as did the controls indicating no effect of an inhibiting factor. The worms subjected to fowl mucus 54 days old gave evidence of some retardation of growth, 17.9 mm as compared with 21.7 mm for the control worms. Gradual increases in retardation of the growth of the worms were demonstrated with fowl mucus up to 70 days old. When 107-day mucus was used, the

TABLE 5.—Showing reactions of *A. galli* cultured in media to which was added autoclaved duodenal mucus from chickens arranged in a graded series from susceptible to resistant hosts

Worms group	Age of mucus (days)	Experimental worms		Controls
		Av. gain (mm)	Av. loss (mm)	Av. gain (mm)
A	45	9.598	...	9.205
B	54	17.916	...	21.740
C	63	14.406	...	17.090
D	66	13.555	...	15.880
E	70	12.090	...	42.380
F	107	4.750	...	28.530
G	117	0.83	8.250
H	125	4.49	9.020

results indicated a marked increase in inhibiting power as the experimental worms gained an average of 4.7 mm as compared with 28.5 mm for the controls. The mucus from chickens 117 and 125 days old, respectively, showed a strong retarding effect by causing the worms to lose length while the controls were gaining 8.2 and 9.0 mm, respectively.

While the results in these various tests were indicative of a more potent growth inhibiting factor in the duodenal mucus of older chickens than in younger ones, the numbers of worms were not sufficient for statistical treatment. It was therefore determined to arrange for an extensive series of tests which would give comparisons of worms of different ages subjected to mucus from chickens of various ages. In these tests the duodenal mucus was not autoclaved; in other respects the tests were essentially similar to the previous ones, namely, that the mucus was introduced in the ratio of 3 cc to 65 cc of nutrient medium and all cultures were adjusted to the same pH of approximately 6.7. *A. galli* from 30 to 90 days of age taken by 10-day intervals were treated with mucus from chickens 60 to 140 days of age considered by 20-day intervals.

The data in this series of experiments when treated by analysis of variance⁶ yielded the highly significant F value of 6.35. It has thus been demonstrated that the duodenal mucus samples differed significantly in the degree of nematode growth inhibition in vitro, and that the effectiveness of the mucus as an inhibitor varied directly with the age of the chickens from which the mucus was taken (Frick and Ackert, 1941). Complete details of these results and of the statistical studies are given by Frick (1941).

Since this substance is present in mucus that has been autoclaved at temperatures that ordinarily destroy antibodies, the explanation of increased natural resistance with age to intestinal worms does not lend itself to an antigen-antibody hypothesis. The host body through its intestinal goblet cells appears to elaborate a thermo-stable substance that is harmful to the helminths.

In tests recently completed by Eisenbrandt and Ackert (1941), it was found that duodenal mucus extracts of adult dogs and hogs caused the early death (24 to 72 hours) of all fowl nematodes (*A. galli*) tested in vitro; whereas, worms in control nutrient solutions lived for a week.

SUMMARY

Evidence from experimental results and epidemiological data indicates that the degree of natural resistance of a host to its helminth parasites is dependent largely upon its diet, its genetic constitution and its age. Diets of man and other omnivorous animals, to serve in developing

⁶ Indebtedness is acknowledged to Dr. H. C. Fryer, Department of Mathematics, Kansas State College, Manhattan, Kansas, for counsel on the statistical treatment of data.

the most potent resistance, should include in the balanced ration, an adequate amount of vitamins A, B (complex), and D, suitable minerals and a range of proteins as supplied in cereals, meat, and milk. Such a diet taken from the young to the adult condition will ordinarily result in the development of a high natural resistance to helminthic infections. By selection it appears to be possible to alter the genetic constitutions in succeeding generations of animals in such manner as to increase their natural resistance to parasites. Just what the nature of the natural resistance is has not been completely elucidated; but there are indications that duodenal mucus from goblet cells contains a thermostable substance that inhibits the development of intestinal helminths.

BIBLIOGRAPHY

- ACKERT, J. E. 1925 Effect of repeated bleeding upon resistance of chickens to parasitism. *J. Parasitol.* 12: 163.
- 1927 Some intestinal worms of chickens and their control. Rep. Proc. 3 World's Poultry Cong. (Ottawa, Canada) pp. 333-336.
- 1931 The morphology and life history of the fowl nematode *Ascaridia lineata* (Schneider). *Parasitology* 23: 360-379.
- ACKERT, J. E. AND BEACH, T. D. 1933 Resistance of chickens to the nematode, *Ascaridia lineata*, affected by dietary supplements. *Tr. Am. Micr. Soc.* 52: 51-58.
- ACKERT, J. E. AND EDGAR, S. A. 1938 Goblet cells and age resistance to parasitism. *J. Parasitol.* 24 Suppl.: 13-14.
- ACKERT, J. E., EDGAR, S. A. AND FRICK, L. P. 1939 Goblet cells and age resistance of animals to parasitism. *Tr. Am. Micr. Soc.* 58: 81-89.
- ACKERT, J. E. AND EISENBRANDT, L. L. 1935 Comparative resistance of bronze turkeys and white leghorn chickens to the intestinal nematode *Ascaridia lineata* (Schneider). *J. Parasitol.* 21: 200-204.
- ACKERT, J. E., EISENBRANDT, L. L., GLADING, B. AND WILMOTH, J. H. 1933 On the comparative resistance of six breeds of chickens to the nematode *Ascaridia lineata* (Schneider). *J. Parasitol.* 20: 127.
- ACKERT, J. E., EISENBRANDT, L. L., WILMOTH, J. H., GLADING, B. AND PRATT, I. 1935 Comparative resistance of five breeds of chickens to the nematode *Ascaridia lineata* (Schneider). *J. Agric. Research, U. S. Dept. Agric.* 50: 607-624.
- ACKERT, J. E., FISHER, MARIAN L. AND ZIMMERMAN, NAOMI B. 1927 Resistance to parasitism affected by the fat-soluble vitamin A. *J. Parasitol.* 13: 219-220.
- ACKERT, J. E. AND HERRICK, C. A. 1928 Effects of the nematode *Ascaridia lineata* (Schneider) on growing chickens. *J. Parasitol.* 15: 1-13.
- ACKERT, J. E., MCILVAINE, MARIAN FISHER AND CRAWFORD, NAOMI ZIMMERMAN 1931 Resistance of chickens to parasitism affected by vitamin A. *Am. J. Hyg.* 13: 320-336.
- ACKERT, J. E. AND NOLF, L. O. 1929 New technique for collecting intestinal roundworms. *Science* 70: 310-311.
- AND ——— 1931 Resistance of chickens to parasitism affected by vitamin B. *Am. J. Hyg.* 13: 337-344.
- ACKERT, J. E., PORTER, D. A. AND BEACH, T. D. 1935 Age resistance of chickens to the nematode *Ascaridia lineata* (Schneider). *J. Parasitol.* 21: 205-213.
- ACKERT, J. E., PRATT, IVAN AND FREEMAN, A. E., JR. 1936 Resistant and susceptible groups of white leghorn chickens to the nematode *Ascaridia lineata* (Schneider). *Anat. Rec.* 67(1 Suppl): 130.

- ACKERT, J. E. AND REID, W. M. 1937 Age resistance of chickens to the cestode *Rallietina cesticillus* (Molin). *J. Parasitol.* 23: 558.
- ACKERT, J. E. AND SPINDLER, L. A. 1929 Vitamin D and resistance of chickens to parasitism. *Am. J. Hyg.* 9: 292-307.
- ACKERT, J. E., TODD, A. C. AND TANNER, W. A. 1938 Growing larval *Ascaridia lineata* (Nematoda) in vitro. *Tr. Am. Micr. Soc.* 57: 292-296.
- ACKERT, J. E. AND WHITLOCK, J. H. 1935 Studies on ascarid nutrition. *J. Parasitol.* 21: 428.
- ACKERT, J. E., WHITLOCK, J. H. AND FREEMAN, A. E., JR. 1940 The food of the fowl nematode, *Ascaridia lineata* (Schneider). *J. Parasitol.* 26: 17-32.
- ACKERT, J. E. AND WILMOTH, J. H. 1934 Resistant and susceptible strains of white minorca chickens to the nematode, *Ascaridia lineata* (Schneider). *J. Parasitol.* 20: 323-324.
- AFRICA, CANDIDO M. 1931 Studies on the host relations of *Nippostrongylus muris*, with special reference to age resistance and acquired immunity. *J. Parasitol.* 18: 1-13.
- AMEEL, DONALD J. 1934 Paragonimus, its life history and distribution in North America and its taxonomy (Trematoda: Troglorematidae). *Am. J. Hyg.* 19: 279-317.
- BAHRS, A. M. 1931 The modification of the normal growth-promoting power for planarian worms, of the digestive mucosa of the rabbit under variations in diet, fasting and age. *Physiol. Zool.* 4: 189-203.
- CABLE, RAYMOND M. 1937 The resistance of the herring gull, *Larus argentatus*, to experimental infections of the trematode, *Parorchis acanthus*. *J. Parasitol.* 23: 559.
- CAMERON, THOMAS W. M. 1935 Immunity against animal parasites. Twelfth Internat. Vet. Cong. 3: 44-65.
- CARREL, ALEXIS AND EBELING, ALBERT H. 1921 Age and multiplication of fibroblasts. *J. Exper. Med.* 34: 599-623.
- CHANDLER, ASA C. 1932a Susceptibility and resistance to helminthic infections. *J. Parasitol.* 18: 135-152.
- 1932b Experiments on resistance of rats to superinfection with the nematode, *Nippostrongylus muris*. *Am. J. Hyg.* 16: 750-782.
- CLAPHAM, PHYLLIS A. 1933 On the prophylactic action of vitamin A in helminthiasis. *J. Helm.* 11: 9-24.
- 1934a Some observations on the effect of dietary deficiency on infestation of chickens with the nematode *Heterakis gallinae*. *J. Helm.* 12: 123-126.
- 1934b Ascariasis and vitamin A deficiency in pigs. *J. Helm.* 12: 165-176.
- CORT, W. W., OTTO, G. F. AND SPINDLER, L. A. 1930 Investigations on *Ascaris lumbricoides* and the associated intestinal helminths of man in southwestern Virginia. *Am. J. Hyg.* 11: 1-55.
- CRAM, ELOISE B. 1940 Studies on oxyuriasis. XXIV. Comparative findings in the white and negro races. *Proc. Helm. Soc. Washington* 7: 31-35.
- CRAM, ELOISE B. AND REARDON, LUCY 1939 Studies on oxyuriasis. XII. Epidemiological findings in Washington, D. C. *Am. J. Hyg.* 29(Sec. D): 17-24.
- CULBERTSON, J. T. 1938 Recent contributions to the immunology of helminthic infections. *Arch. Path.* 25: 85-117; 256-280.
- 1941 Immunity against Animal Parasites. Columbia Univ. Press, New York. 274 pp.
- CURTIS, M. R., DUNNING, W. F. AND BULLOCK, F. D. 1933 Is malignancy due to a process analogous to somatic mutation? *Science* 77: 175-176.
- DAVIS, DORLAND J. 1936 Pathological studies on the penetration of the cercaria of the strigeid trematode, *Diplostomum flexicaudum*. *J. Parasitol.* 22: 329-337.
- EISENBRANDT, L. L. AND ACKERT, J. E. 1941 Effects of duodenal mucus of dogs and swine upon the viability of *Ascaridia lineata* in vitro. *J. Parasitol.* 27 Suppl.: 36.

- FISHER, A. C. 1934 A study of the schistosomiasis of the Stanleyville district of the Belgian Congo. Tr. Roy. Soc. Trop. Med. and Hyg. 28: 277-306.
- FOSTER, A. O. 1936 On a probable relationship between anemia and susceptibility to hookworm infection. Am. J. Hyg. 24: 109-128.
- FOSTER, A. O. AND CORT, W. W. 1931 The effect of diet on hookworm infestation in dogs. Science 73: 681-683.
- AND ——— 1935 Further studies on the effect of a generally deficient diet upon the resistance of dogs to hookworm infestation. Am. J. Hyg. 21: 302-318.
- FRICK, L. P. 1941 Studies on a mechanism of age resistance of chickens to the nematode *Ascaridia galli*. Unpublished thesis. Kansas State Coll. Agric. and Appl. Sc. 54 pp.
- FRICK, L. P. AND ACKERT, J. E. 1941 The role of duodenal mucus in age resistance. J. Parasitol. 27 Suppl: 36-37.
- GRAHAM, G. L. 1934 Resistance studies with the nematode, *Nippostrongylus muris*, in laboratory rats. Am. J. Hyg. 20: 352-372.
- GRAHAM, G. L. AND PORTER, DALE A. 1934 Strains of *Nippostrongylus muris* and their behavior in various strains of rats. J. Parasitol. 20: 323.
- GREGORY, P. W. 1937 The possibility of establishing within breeds lines of sheep that are genetically resistant to stomach worms. Am. Soc. Animal Prod., Rec. Proc. Am. Meet. pp. 316-324.
- GREGORY, P. W., MILLER, R. F. AND STEWART, M. A. 1940 An analysis of environmental and genetic factors influencing stomach-worm infestation in sheep. J. Genetics 39: 391-400.
- HERRICK, C. A. 1926 Studies on the resistance of chickens to the nematode *Ascaridia perspicillum*. Am. J. Hyg. 6: 153-172.
- 1928 A quantitative study of infections with *Ancylostoma caninum* in dogs. Am. J. Hyg. 8: 125-157.
- HIRAISHI, T. 1926 [Experimental ascariasis of the young pigs with special reference to A-avitaminosis.] Keio Igaku, Tokyo (J. Keio Med. Soc.) 6. Summary in Japan Med. World, 1927, 7: 79-80.
- KAUZAL, G. 1934 Observations on the development of resistance to *Dictyocaulus filaria*. Austral. Vet. J. 10: 100-111.
- KELLER, A. E., LEATHERS, W. S. AND BISHOP, E. L. 1932 A state wide study of the human intestinal helminths in Tennessee. J. Prev. Med. 6: 161-184.
- KELLER, A. E., LEATHERS, W. S. AND KNOX, J. C. 1937 The present status of hookworm infestation in North Carolina. Am. J. Hyg. 26: 437-454.
- KELLER, A. E., LEATHERS, W. S. AND RICKS, H. C. 1934 An investigation of the incidence and intensity of infestation of hookworm in Mississippi. Am. J. Hyg. 19: 629-656.
- LAPAGE, GEOFFREY 1937 Nematodes Parasitic in Animals. Methuen & Co., Ltd., London. 172 pp.
- LAWLER, H. J. 1941 The relation of vitamin A to immunity to Strongyloides infection. Am. J. Hyg. 34(Sec. D): 65-72.
- LEATHERS, W. S., KELLER, A. E. AND MCPHAUL, W. A. 1939 An investigation concerning the status of hookworm in Florida. Am. J. Hyg. 29(Sec. D): 1-16.
- LOOSS, A. 1911 The Anatomy and Life-history of *Agchylostoma duodenale* Dub. Rec. Sch. Med., Egypt, Min. Educ., Cairo 4: 163-613.
- LUTTERMOSER, GEORGE W. 1938 An experimental study of *Capillaria hepatica* in the rat and the mouse. Am. J. Hyg. 27: 321-340.
- MCCOY, O. R. 1934 The effect of vitamin A deficiency on the resistance of rats to infection with *Trichinella spiralis*. Am. J. Hyg. 20: 169-180.
- MILLER, J. D. 1908 The government veterinary surgeon's report on the disease caused by the Strongylus parasite. Proc. Agric. Soc. Trinidad and Tobago 8: 373-375.

- NOLF, L. O. AND ZAIMAN, HERMAN 1941 The effect of host age on the number of *Trichinella spiralis* recovered from rats during the early period of infection. J. Parasitol. 27 Suppl: 24.
- OTTO, G. F. 1936 Human infestation with the dwarf tapeworm (*Hymenolepis nana*) in the southern United States. Am. J. Hyg. 23: 25-32.
- OTTO, G. F. AND CORT, W. W. 1934 The distribution and epidemiology of human ascariasis in the United States. Am. J. Hyg. 19: 657-712.
- OTTO, G. F. AND LANDSBERG, J. W. 1940 Dietary deficiencies and iron salts in hookworm infections. Am. J. Hyg. 31(Sec. D): 37-47.
- PAYNE, FLORENCE K., ACKERT, J. E. AND HARTMAN, ERNEST 1925 The question of the human and pig ascaris. Am. J. Hyg. 5: 90-101.
- PORTER, DALE A. 1935a A comparative study of *Nippostrongylus muris* in rats and mice. Am. J. Hyg. 22: 444-466.
- 1935b Studies on the effect of milk diet on the resistance of rats to *Nippostrongylus muris*. Am. J. Hyg. 22: 467-474.
- PORTER, DALE A. AND ACKERT, J. E. 1933 The effect of blood loss upon the resistance of chickens to variable degrees of parasitism. Am. J. Hyg. 17: 252-261.
- RANSOM, B. H. 1921 The turkey an important factor in the spread of gapeworm. Bull. U. S. Dept. Agric. 939: 1-13.
- RANSOM, B. H. AND FOSTER, W. D. 1920 Observations on the life history of *Ascaris lumbricoides*. Bull. U. S. Dept. Agric. 817: 1-47.
- REID, W. M. 1940 Some effects of short starvation periods upon the fowl cestode *Railletina cesticillus* (Molin). J. Parasitol. 26 Suppl: 16.
- ROBERTS, F. H. S. 1937 Studies on the biology and control of the large roundworm of fowls *Ascaridia galli* (Schränk, 1788) Freeborn, 1923. Bull. Animal Health Sta., Yeerongpilly, Dept. Agric. and Stock, Queensland 2: 1-106.
- 1939 The gastro-intestinal helminths of cattle in Queensland: their distribution and pathogenic importance. Proc. Roy. Soc. Queensland 50: 46-54.
- SANDGROUND, J. H. 1929 A consideration of the relation of host-specificity of helminths and other metazoan parasites to the phenomena of age resistance and acquired immunity. Parasitology 21: 227-255.
- SARLES, MERRITT P. 1929a Quantitative studies on the dog and cat hookworm, *Ancylostoma braziliense*, with special emphasis on age resistance. Am. J. Hyg. 10: 453-475.
- 1929b The reaction and susceptibility of dogs of different ages to cutaneous infection with the dog hookworm, *Ancylostoma caninum*. Am. J. Hyg. 10: 683-692.
- SCHWARTZ, B., ALICATA, J. E. AND LUCKER, J. T. 1931 Resistance of rats to superinfections with a nematode, *Nippostrongylus muris*, and an apparently similar resistance of horses to superinfection with nematodes. J. Wash. Acad. Sc. 21: 259-261.
- SCOTT, J. ALLEN 1928 An experimental study of the development of *Ancylostoma caninum* in normal and abnormal hosts. Am. J. Hyg. 8: 158-204.
- SHORB, DOYS ANDREW 1933 Host-parasite relations of *Hymenolepis fraterna* in the rat and the mouse. Am. J. Hyg. 18: 74-113.
- SMILLIE, W. G. 1922 Studies on hookworm infestation in Brazil. Monogr. Rockefeller Inst. Research 17: 1-73.
- SMILLIE, W. G. AND AUGUSTINE, D. L. 1925 Intensity of hookworm infestation in Alabama. J. Am. Med. Assn. 85: 1958-1963.
- STEWART, M. A., MILLER, R. F. AND DOUGLAS, J. R. 1937 Resistance of sheep of different breeds to infestation by *Ostertagia circumcincta*. J. Agric. Research, U. S. Dept. Agric. 55: 923-930.
- STOLL, NORMAN R. 1929 Studies with the strongyloid nematode, *Haemonchus contortus*. I. Acquired resistance of hosts under natural reinfection conditions out-of-doors. Am. J. Hyg. 10: 384-418.

- TALIAFERRO, W. H. 1929 The Immunology of Parasitic Infections. Century Co., New York 414 pp.
- TAYLOR, E. L. 1934 Field experiments on the immunity of lambs to parasitic gastritis caused by a mixed infection of trichostrongylid nematodes. J. Helm. 12: 143-164.
- WINFIELD, GERALD F. 1933 Quantitative experimental studies on the rat nematode *Heterakis spumosa*, Schneider, 1866. Am. J. Hyg. 17: 168-228.
- WRIGHT, WILLARD H. 1935 The relation of vitamin A deficiency to ascariasis in the dog. J. Parasitol. 21: 433.
- ZAIMAN, HERMAN 1940 The effect of host vitamin E deficiency on *Trichinella spiralis* infections. J. Parasitol. 26 Suppl: 44.
- ZIMMERMAN, NAOMI B., VINCENT, LOLA B. AND ACKERT, J. E. 1926 Vitamin B, a factor in the resistance of chickens to *Ascaridia perspicillum* (Rud.). J. Parasitol. 12: 164.

FIVE NEW SPECIES OF AVIAN SCHISTOSOMES FROM WIS-
CONSIN AND MICHIGAN WITH THE LIFE CYCLE OF
GIGANTOBILHARZIA GYRAULI (BRACKETT,
1940)*

STERLING BRACKETT

University of North Carolina, School of Public Health, Chapel Hill

In a recent summary of the distribution of schistosome dermatitis in this country (Brackett, 1941), it was reported that the disease is to be found chiefly in the northern parts of Michigan, Wisconsin, Minnesota, and Manitoba. In this area, *Cercaria stagnicola* Talbot, 1936, is responsible for the largest proportion of the outbreaks. This can partially be explained by the fact that its snail hosts, varieties of *Stagnicola emarginata*, prefer a habitat that is also chosen by bathers and that this snail species reaches the height of its development during the resorting season. Within this important center of schistosome dermatitis, the disease was found to be distributed unequally and to fluctuate from year to year. Neither of these features could be completely correlated with the presence or absence or fluctuations of the snail hosts and it was suggested that the explanation probably would be found when the life cycle of *C. stagnicola* is demonstrated and the adult stage and its host become known. Talbot (1936) and others have attempted to determine the life cycle of *C. stagnicola* by exposing birds and mammals to this form. Perhaps these experiments have all been unsuccessful because this cercaria may be very specific in its definitive host and the proper bird or mammal might not have been used. In an effort to become better acquainted with the adult schistosome fauna of the dermatitis areas McLeod (1940) and the author (Brackett, 1940a) and others have examined many birds and some mammals from Manitoba, Wisconsin, and Michigan specifically for blood flukes. The results of these investigations combined with reports in the literature showed that about 10 species of schistosomes occurred in birds to be found in Wisconsin and vicinity. The present paper reports the results of the examination of birds in the spring and summer of 1940 in Wisconsin and Michigan during which time 5 previously undescribed schistosomes were found.

The principal difficulty in the taxonomy of the avian schistosomes is that many of the known forms are described incompletely due to the in-

Received for publication, February 12, 1941.

* A contribution from the Department of Zoology, University of Wisconsin; the Biological Station of the University of Michigan; and the School of Public Health, University of North Carolina. Aided in part by a grant from the Wisconsin Alumni Research Foundation.

The advice and encouragement of Dr. C. A. Herrick is gratefully acknowledged.

adequacy of the material. There is probably no trematode group in which it is more difficult to get suitable specimens for study. The avian schistosomes are often very minute and extremely fragile and, therefore, practically impossible to extricate if they are in the smaller veins overlying the intestine. In addition, since the schistosomes are diecious, it is necessary to obtain specimens of both sexes for a complete specific description. This has often not been possible. Because the descriptions of the species have so frequently been incomplete, and because there are unquestionably many species yet unknown, it is obviously difficult to define and delimit the genera of the family SCHISTOSOMATIDAE. In view of these facts the species to be described in this paper are placed in existing genera with no effort made to modify the generic conceptions. It is believed that any further attempt to revise the schistosome genera should be withheld until the species have been more clearly defined and until the group is more completely known.

In contrast to the conservatism regarding the genera the author has tended to go to extremes in splitting species. The principal reason for this is to avoid any chance of building up a species complex which might later complicate the solution of the life cycles of these forms. Talbot, by very careful and painstaking studies, cleared up a species complex of schistosome cercariae by demonstrating that what had been considered a single species, *C. elvae* Miller, 1923, actually represented three species very similar in morphology. There is no reason to believe that the adults of these cercariae should not likewise be similar and easily confused. If life cycle experiments show that more than one of the species of adults herein described develop from a single species of cercaria, it will cause very little confusion to drop some of the forms into synonymy. On the other hand if several similar adults were thrown together as a single species, it would only be with great difficulty that they could later be conclusively separated.

METHODS

In all cases the birds were either shot or trapped by the author. Because it is desirable to make the examinations as soon as possible after the death of the host many of them were examined in the field. This is difficult but so important that it is well worth the effort required to get the necessary apparatus into the field. Ordinarily a make-shift laboratory was set up in an automobile and the car driven as close to the collecting areas as feasible. If the worms were found in some of the larger veins, these vessels were tied off and removed to saline before being opened. If the worms were in the smaller mesenteric veins, the mesenteries were stripped from the organs and placed in saline on a glass plate. With fine instruments the worms were then dissected from the veins. At any event the cloaca and large intestine and other sections of the intestine were split and

laid out on a glass plate so that the vessels on their surfaces could be examined with the dissecting microscope. This is a very important step in detecting the smaller forms or females and no examination is complete without this procedure. Worms seen in the minute veins on the intestinal wall were best recovered by cutting across the vessel close to the specimen and then gently forcing the worms out by lightly stroking the vessel with a fine but blunt instrument. The worms were transferred to saline and then dropped into Gilson's fixative at 60° C. In most cases, the worms relaxed reasonably well with this treatment although the filamentous forms never completely straightened out. Staining results varied with each species but alum-cochineal yielded the most consistently good specimens. Semichon's carmine frequently gave good results.

THE GENUS *Pseudobilharziella*

Some very minute blood flukes were found in four of the species of ducks examined in Wisconsin. These worms were all in the small veins on the surface of the large intestine and cloaca. Examination of stained specimens as well as observations on living specimens showed that all of these worms are very similar in morphology but it is possible to separate them into four forms which are here described as separate species. These worms are closely related and fit readily into the genus *Pseudobilharziella* as defined by Ejsmont (1929) since they are filamentous, have both oral and ventral suckers well developed, have a short but distinct gynaecophoric canal, and have the anterior part of the body much shorter and somewhat wider than the long posterior part which contains the numerous testes.

Pseudobilharziella waubesensis n. sp.

(Figs. 1-2)

Specific description: Pseudobilharziella: Filamentous, cylindrical worms up to 5.62 mm in length. Both sexes reasonably similar in size and shape but female slightly wider than the section of the male behind the gynaecophoric canal. Width of body of female uniformly about 0.047 mm. Width of male about 0.047 to 0.062 mm at acetabulum, from 0.072 to 0.082 mm at gynaecophoric canal, and about 0.032 mm behind the gynaecophoric canal. Both oral and ventral suckers well developed.

Male: Acetabulum about 0.040 mm in diameter and about 0.275 mm from the anterior end and with several circles of fine spines. Body finely striated but not spined. Gynaecophoric canal well defined, 0.252 to 0.306 mm in length and about 0.540 mm from anterior end and thickly spined. Testes approximately 100 in number, about 0.035 mm long and 0.018 mm wide and arranged in tandem. Testes separated by a distance equal to or greater than the length of a testis; the body constricts between them to give a beaded appearance to the whole section back of the gynaecophoric canal. Testes extend from posterior end of the gynaecophoric canal to the end of the body. Vas deferens leads forward as a fine tubule to a point just back of the acetabulum where it turns posteriorly and expands into a large seminal vesicle which is coiled into about ten loops. The sperm duct leads directly as a simple tubule to the genital pore at the anterior end of the gynaecophoric canal. Oral sucker about 0.040 mm long and 0.028 mm wide. Mouth slightly subterminal. Simple esophagus bifurcates just cephalad of the acetabulum. Cecal reunion not observed.

Female: Acetabulum 0.306 mm from anterior end. Ovary not coiled, only slightly irregular in its course; about 0.325 mm long and situated 0.684 mm from anterior end of body. Ovary opens almost directly into the seminal receptacle which is about 0.050 mm long. Oviduct arises at posterior end of seminal receptacle, immediately turns anteriorly and proceeds as a small tubule to a point about mid-way between the acetabulum and the anterior tip of the ovary where it expands into the large thin walled oötype. A spindle shaped egg about 0.150 mm in length was enclosed in the oötype of the specimen examined. Its terminal spines extended in either direction beyond the limits of the main body of the oötype. The uterus leads forward from the oötype past the acetabulum but the exact location of the genital pore remains undetermined. The vitellaria consist of a great number of small follicles which start from a point about 0.150 mm caudad of the ovary and lie on both sides of the cecum, extending presumably to the posterior end of the body. Vitelline duct not observed. Simple esophagus bifurcates just cephalad of the acetabulum and the ceca reunite again in the region of the seminal receptacle.

Hosts: *Nyroca collaris* (Donovan) and *Mareca americana* (Gmelin).

Location: Intestinal and cloacal veins.

Locality: Lake Waubesa, near Madison, Wis., U. S. A.

Type specimens: U. S. Nat. Mus. Helm. Coll. No. 44868.

This description is based on detailed studies of 3 complete or nearly complete male specimens and 1 nearly complete female specimen from a baldpate duck, and 1 nearly complete male specimen from a ring-necked duck.

Pseudobilharziella kegonsensis n. sp.

(Fig. 3)

Specific description: *Pseudobilharziella:* *Male:* Long, cylindrical body of uniform diameter except for the slightly enlarged region of the gynaecophoric canal and the expanded posterior tip. Body length about 4.03 mm and maximum body width (excluding gynaecophoric canal region) 0.054 mm. Body surface finely striated but not spined. Oral sucker 0.036 mm long and 0.025 mm wide. Ventral sucker (0.288 mm from anterior end) 0.036 mm in diameter, may protrude noticeably, and bears a number of circles of fine spines. Gynaecophoric canal well defined, 0.144 mm long and situated 0.54 mm from anterior end, and thickly spined. Body in this region 0.65 mm in diameter. Testes about 150 in number, 0.022 mm long and 0.032 mm wide, arranged in tandem and lying close together but usually not contiguous. They extend from posterior margin of the gynaecophoric canal to the posterior tip of the body. The vas deferens leads forward as a fine tubule to the region of the acetabulum where it turns posteriorly and expands into the seminal vesicle. The seminal vesicle is coiled into about 6 loops and extends almost to the gynaecophoric canal. At its posterior end it narrows into a simple, short sperm duct that empties through the genital pore which is on a small papilla at the anterior end of the gynaecophoric canal. The oral opening is slightly ventral in position. The simple esophagus bifurcates into the intestinal ceca about mid-way between the oral and ventral suckers. The point of cecal reunion was not determined.

Female: Unknown. Eggs in intestinal mucosa of host were spindle-shaped.

Host: *Nyroca valisineria* (Wilson).

Location: Cloacal vein.

Locality: Lake Kegonsa, near Madison, Wis., U. S. A.

Type specimen: U. S. Nat. Mus. Helm. Coll. No. 44865.

This description is based on a detailed study of a single, complete male specimen stained with Semichon's carmine stain. Several worms were seen at autopsy of the one infected canvas-back duck but it was possible

to recover only one of them. The mucosa of the large intestine and cloaca of this bird contained numerous spindle shaped eggs. These hatched within a few minutes after being placed in tap water and the miracidia proved to be typical of the family SCHISTOSOMATIDAE. The miracidia are about 0.20 mm in length.

Pseudobilharziella horiconensis n. sp.

(Figs. 4a and 6)

Specific description: Pseudobilharziella: Male: Filamentous body about 3.56 mm long which may be divided into three parts: a short section from the anterior end to the gynaecophoric canal which is about 0.057 mm wide; the anterior half of the section back of the gynaecophoric canal which has roughly parallel margins and is about 0.038 mm wide; and the posterior section of the body which is very narrow and constricts between the testes giving a beaded appearance. Body surface finely striated but not spined. Oral sucker 0.036 mm long and 0.032 mm in diameter and possessing several circles of fine spines. Gynaecophoric canal well defined, 0.125 mm long and situated 0.432 mm from anterior end and thickly spined. Body in this region is 0.065 mm wide. Testes not over 115 in number; extend from posterior end of gynaecophoric canal to posterior end of body in tandem arrangement. Those in the anterior section of this body region are so closely arranged they are contiguous while those in the posterior, narrower section of the body may be a considerable distance from each other with the body wall constricting between them. The vas deferens leads forward as a fine tubule to a point just behind the acetabulum where it bends posteriorly and expands into the wide coiled seminal vesicle. The seminal vesicle is coiled into about four to six loops and extends almost to the gynaecophoric canal. At its posterior end it narrows into a simple, short sperm duct that empties through the genital pore which is on a small papilla at the anterior end of the gynaecophoric canal and slightly to the right of the mid-line. The oral opening is terminal or slightly sub-terminal. The simple esophagus bifurcates closer to the ventral than to the oral sucker. The location of the cecal reunion was not determined.

Female: Unknown.

Host: *Nyroca americana* (Eyton).

Location: Cloacal vein.

Locality: Horicon Marsh, near Mayville, Wis., U. S. A.

Type specimen: U. S. Nat. Mus. Helm. Coll. No. 44866.

This description is based on a detailed study of a single, complete male specimen stained with Semichon's stain.

Pseudobilharziella burnetti n. sp.

(Fig. 5)

Specific description: Pseudobilharziella: Male: Long, cylindrical body of uniform diameter except for the slightly enlarged region of the gynaecophoric canal. Posterior tip not seen. Length of body not determined; body width about 0.048 mm. Body surface finely striated but not spined. Oral sucker wider than long; 0.032 mm long and 0.036 mm wide. Ventral sucker about 0.216 mm from anterior end and 0.040 mm in diameter, and has several circles of fine spines. Gynaecophoric canal well defined; 0.144 mm long and situated 0.45 mm from anterior end, and is thickly spined. Body in this region 0.065 mm in diameter. Number of testes not determined. Testes wider than long; arranged in tandem and contiguous with each other; extending from the posterior margin of the gynaecophoric canal presumably to the caudal end of the body. The vas deferens leads forward as a fine tubule to a point mid-way between the acetabulum and the gynaecophoric canal where it bends

posteriorly and expands into a short, wide seminal vesicle which is coiled in three to four loops. At its posterior end the seminal vesicle narrows into a simple, short sperm duct that empties through the genital pore on the right side of the anterior end of the gynaecophoric canal. The oral opening is sub-terminal. The simple esophagus bifurcates into the intestinal ceca just in front of the acetabulum. The point of cecal reunion was not determined.

Female: Unknown.

Host: *Nyroca collaris* (Donovan).

Location: Cloacal vein.

Locality: Horicon Marsh near Burnett, Wis., U. S. A.

Type specimen: U. S. Nat. Mus. Helm. Coll. No. 44867.

The description is based on a detailed study of a single, male specimen in two fragments stained with Semichon's carmine stain.

These new species are differentiated one from the other as well as from previously described members of the genus in the following key. This key is based only on the males because, with the exception of *P. waubesensis*, the females are either unknown or inadequately described. Apparently the males of this group are more apt to be found.

KEY TO MALES OF THE GENUS *Pseudobilharziella*

1. Testes on each side of common cecum; cecum usually convoluted . . . 2
 Testes arranged linearly in tandem; common cecum more or less straight 4
2. Testes numerous, more than one in each cross section 3
 Usually only one testis in each cross section; these lie alternately on each side of cecum . . . *Pseudobilharziella yokogawai* (Oiso, 1927)
3. Less than 4 mm in length; genital pore anterior to cecal union.
 Pseudobilharziella querquedulae McLeod, 1937
 More than 5 mm in length; genital pore posterior to cecal union.
 Pseudobilharziella kowalewskii Ejsmont, 1929
4. Larger worms, 11–12 mm in length and 0.15 mm in width; testes large, 0.08 mm by 0.06 mm.
 Pseudobilharziella filiformis Szidat, 1939
 Smaller worms, less than 5–6 mm in length and very slender 5
5. Body cylindrical and uniform in diameter 6
 All or part of body back of gynaecophoric canal constricted between testes to give a beaded appearance 7
6. Seminal vesicle only one-half the length of the distance from the acetabulum to gynaecophoric canal.
 Pseudobilharziella burnetti, n. sp.
 Seminal vesicle long, stretching from acetabulum to gynaecophoric canal *Pseudobilharziella kegonsensis* n. sp.
7. Gynaecophoric canal over 0.25 mm long; seminal vesicle coiled in about 10 loops, extending from acetabulum to gynaecophoric canal.
 Pseudobilharziella waubesensis n. sp.

Gynaecophoric canal about 0.150 mm long; seminal vesicle wide and forming only 4-6 loops *Pseudobilharziella horiconensis*, n. sp.

If the new species described here are not in the future proved to constitute a single species, then the genus *Pseudobilharziella* rivals the genus *Schistosoma* in size. The eight species now placed in the genus *Pseudobilharziella* seem to form a rather homogeneous group as far as can be determined from the yet inadequate material available. In addition they are all found in ducks with the exception of *P. filiformis* which occurs in the swan. Males of all eight species have been described. In *P. querquedulae* and *P. filiformis* a cirrus sac and related organs and glands are reported. No reference is made to these structures in *P. kowalewskii* or *P. yokogawai*. Although these structures were not seen in the new species even after careful study, it is still possible that they might be present. It is the author's opinion that a cirrus and cirrus sac would be found to exist if more and better prepared specimens were available. In the males of all eight species the testes are numerous, completely filling the body caudad of the gynaecophoric canal; a seminal vesicle is prominent, loosely coiled and generally fills the region between the intestinal ceca; the genital pore is situated at the anterior end of the gynaecophoric canal; and the gynaecophoric canal is short but prominent, situated near the anterior end of the body and set with numerous fine spines.

In the literature the females of *P. filiformis* and *P. yokogawai* have been reported. Those of the former species are smaller than the males while those of the latter species are longer although more slender than the males. Nothing concerning the structure of these females is available. The author has seen females of *P. querquedulae* but has been unable to obtain specimens suitable for study. All that can be said is that they are much smaller than the males. Thus it is seen that the description of the female of *P. waubensis* adds considerably to knowledge of the genus *Pseudobilharziella*. The sexes of this species are similar in general size and appearance. The ovary is in the anterior end of the worm. It opens into a fairly large seminal receptacle at its posterior end which in turn opens into the oviduct leading forward from this point. About mid-way between the ovary and the acetabulum, the oviduct expands into a large thin-walled oötype. The uterus leads forward past the acetabulum but the location of the genital pore is not determined. The vitellaria consist of numerous small follicles that completely fill the body back of the seminal receptacle.

The eggs of *P. filiformis* and *P. yokogawai* have been described in the literature and those of *P. waubensis*, *P. kegonsensis*, and *P. querquedulae* have been seen by the author. In all cases except of *P. filiformis* the eggs are spindle-shaped with a long blunt spine on either end. The

egg of *P. filiformis* is semi-oval with only a very tiny spine at one end. In all cases the eggs contain fully developed miracidia when passed from the host and these hatch within a few minutes after reaching water.

The life cycle of *P. yokogawai* has been described by Oiso (1927). He found in Formosa that cercariae very similar to the typical dermatitis-producing schistosome cercariae of North America would penetrate the skin of ducks and develop in about two weeks into mature schistosomes in the small veins of the lower gut. Miracidia which hatched from the spindle shaped eggs of this species penetrated into lymnaeid snails. No life cycles of other members of the genus have been demonstrated experimentally but upon epidemiological evidence the author (Brackett, 1940c) suggested that the larval stage of *P. querquedulae* may prove to be *C. physellae* which develops in *Physa* snails in Wisconsin and Michigan. It seems not unlikely then that subsequent experiments will show that some of the new species of *Pseudobilharziella* described here develop from some of the cercariae involved in outbreaks of dermatitis.

THE GENUS *Gigantobilharzia*

In certain restricted regions in Wisconsin a large percentage of the red-winged and yellow-headed blackbirds were found to harbor a long filamentous blood fluke in the tiny veins encircling the small intestine. The intestines of these worms often were packed with dark material which made them quite conspicuous especially under magnification. While large numbers of these worms were seen, very few specimens suitable for study could be removed since their great length prevented their being pushed or pulled out of the vessels.

These worms have been placed in the genus *Gigantobilharzia* and since, as will be presently described, the miracidia were shown after development in snails to give rise to *Cercaria gyrauli* Brackett, 1940, this species then becomes *Gigantobilharzia gyrauli* (Brackett, 1940) n. comb.

Gigantobilharzia gyrauli (Brackett, 1940)

(Figs. 6-7)

Specific description: *Gigantobilharzia*: Extremely filamentous. Sexes similar in size and shape. Body cylindrical, about 10 mm in length with posterior tip bluntly rounded. Body width uniformly about 0.045 to 0.050 mm. Oral sucker well developed and 0.030 mm long. Ventral sucker absent.

Male: Gynaecophoric canal present; 0.8 mm in length and 1.38 mm from anterior tip of body. Body 0.054 mm wide at gynaecophoric canal. Testes very numerous; distance between each testis equal to or greater than diameter of each follicle. They extend from a point about 0.26 mm back of gynaecophoric canal to posterior tip of body. Vas deferens leads forward from testes as a very fine tubule to a point about 0.28 mm from anterior end of body where it bends posteriorly and expands into the seminal vesicle. Seminal vesicle about 0.26 mm long and coiled in 8 to 10 loops. Sperm duct leads directly as a plain tubule to the genital pore which is situated on a small papilla at anterior end of gynaecophoric canal.

Female: Ovary coiled in about 10 loops; 0.45 mm in length extending posteriorly

from a point 1.4 mm from anterior tip of body. Posterior end of ovary narrows into a tubule 0.018 mm in length which leads into a seminal receptacle 0.15 mm in length. Oviduct arises at posterior end of seminal receptacle and immediately turns anteriorly and continues to a point 1.115 mm from anterior end of body where it expands into a large thin walled oötype. The uterus leads forward from the oötype to the genital pore which is situated immediately behind the oral sucker. Vitellaria in numerous follicles closely arranged and practically filling the entire body from a point a short distance caudad of the seminal receptacle to the posterior tip of the body. Vitelline duct leads forward and joins the oviduct just before it enters the oötype. No eggs observed *in utero*. Eggs only slightly elongate, about 0.060 mm long, thin shelled and without spine. Esophagus bifurcates 0.54 mm from anterior end and ceca reunite just caudad of seminal receptacle.

Hosts: *Agelaius phoeniceus arctolegus* Oberholser and *Xanthocephalus xanthocephalus* (Bonaparte).

Location: Intestinal veins.

Locality: Sun Prairie, Wis., U. S. A.

Type specimens: U. S. Nat. Mus. Helm. Coll. No. 44864.

The description of this species is based on detailed studies of fragments of four males and four females and general observations on many more fragments. The worms were collected from 20 red-winged and 5 yellow-headed blackbirds.

This species differs from *G. acotylae* Odhner, 1910 in being much smaller, in having a better developed oral sucker, and in having a longer gynaecophoric canal situated farther back from the anterior end. It is unlike both *G. egretta* Lal, 1937 and *G. monocotylea* Szidat, 1930 since neither of these possess a gynaecophoric canal.

Herring and ring-billed gulls in the region of the Biological Station of the University of Michigan at Douglas Lake, Michigan, were found to harbor *Microbilharzia lari* (McLeod, 1937). In addition some of these birds were found to harbor what proved to be an undescribed form which has also been placed in the genus *Gigantobilharzia*. These worms are reasonably large and were most frequently found in the larger intestinal veins and hepatic portal vein.

Gigantobilharzia lawayi n. sp.

(Figs. 8-9)

Specific description: *Gigantobilharzia*: Extremely filamentous. Female as long as or longer than male and similar in shape but more slender.

Male: About 7.3 mm in length and 0.150 mm in diameter at gynaecophoric canal and 0.105 mm elsewhere. Gynaecophoric canal present, anterior end distinct but posterior end terminates obscurely; 0.63 to 0.85 mm from anterior end and may be as long as 2.25 mm or may be shorter. Testes over 500 in number, wider than long, and arranged in tandem and usually close together. Testes extend from posterior tip of body to a point further forward than the posterior end of gynaecophoric canal but not beyond its equator. Vas deferens leads forward as a fine tubule to a point from 0.37 to 0.52 mm from the anterior end where it bends posteriorly and expands into the seminal vesicle. Seminal vesicle 0.30 to 0.37 mm in length and coiled in about six or seven loops. Cirrus and cirrus sac present. Genital pore situated at anterior end of gynaecophoric canal. Intestine bifurcates from 0.22 to 0.30 mm from anterior end. Point of cecal reunion not seen.

Female: About 10 mm in length and a maximum width of 0.060 mm. Ovary coiled in 8 to 10 loops; 0.22 mm in length and situated 0.71 mm from anterior end.

Posterior end of ovary opens almost directly into seminal receptacle which is 0.075 mm in length. Oviduct arises at posterior end of seminal receptacle and immediately turns anteriorly and proceeds to a point 0.60 mm from anterior end where it expands into a large thin-walled oötype. The uterus runs forward from the oötype and the genital pore is probably just behind the oral opening. Vitellaria small and numerous and start a short distance posterior to the seminal receptacle and fill the body caudad of this point. Vitelline duct not observed. No eggs seen in the uterus of the worms or in the intestinal mucosa of the hosts. Digestive system not observed.

Hosts: *Larus argentatus smithsonianus* Coues and *L. delawarensis* Ord.

Location: Intestinal veins and hepatic portal.

Locality: Douglas Lake, Michigan, U. S. A.

Type specimens: U. S. Nat. Mus. Helm. Coll. Nos. 44862 and 44863.

The description of this species is based on detailed studies of 4 entire or almost entire male specimens and one almost entire female. Reference to the following key to the males of the genus *Gigantobilharzia* will serve to differentiate this species from others of the genus.

KEY TO MALES OF THE GENUS *Gigantobilharzia*

1. Gynaecophoric canal present, more or less well developed 2
 Gynaecophoric canal absent 4
2. Oral sucker present 3
 Oral sucker absent *Gigantobilharzia lawayi* n. sp.
3. 140 to 165 mm long and 0.25 to 0.35 mm wide.

Gigantobilharzia acotylea Odhner, 1910

 About 10 mm long and 0.06 mm wide.

Gigantobilharzia gyrauli (Brackett, 1940)

4. Oral sucker present *Gigantobilharzia monocotylea* Szidat, 1930
 Oral sucker absent *Gigantobilharzia egreta* Lal, 1937

The genus *Gigantobilharzia* is difficult to define precisely because the species now referred to it may not be cogenetic; but because the group is not yet well enough known a revised generic diagnosis is not proposed even though Odhner's (1910) characterization of the genus is now inadequate. However, it seems worth while to call attention to several points. None of the species possess an acetabulum although an oral sucker may or may not be present. A gynaecophoric canal is absent in some species and poorly defined in others. The testes, at least in some species (*G. lawayi* and *G. egreta*), are more numerous than in any other known forms. The eggs are known for 3 species and all are oval without spines or with only a tiny spine at one end. In addition, as contrasted with the genus *Pseudobilharziella*, these species are not found in ducks but in gulls, blackbirds and the cattle egret of India.

THE LIFE CYCLE OF *Gigantobilharzia gyrauli*

Usually the mucosa of the intestine of infected blackbirds was found to contain large numbers of the eggs of *G. gyrauli*. Many of these eggs

contained fully developed miracidia and it is assumed that they are in this condition when passed from the host. Eggs scraped from the mucosa were put in tap water where some hatched within a few minutes and others continued to hatch over a period of two days.

The miracidium has two flame cells on each side of the body and in other respects is characteristic of this stage of species of the family SCHISTOSOMATIDAE. It is considerably larger than the miracidium of the human schistosomes, however, since it is 0.20 mm in length as compared with a length of about 0.13 mm for the miracidium of *Schistosoma haematobium*. The large size of the miracidia seems to be a characteristic of the avian schistosomes. The miracidia swim about actively and are attracted towards light.

On May 6, 1940, the following snails *Gyraulus parvus* (Say), *Menetus exacuus* (Say), and a species of *Physa* collected from Williams Creek near Madison, Wisconsin, were put in an aquarium containing water and plants from the habitat of the snails. The aquarium was aerated and kept at room temperature (about 70° F). Miracidia of the blackbird schistosome were introduced into this aquarium on May 6, 8, and 13, 1940. Snails in this experiment were first examined by autopsy on June 6, 1940, with the following results:

- G. parvus*
 - 29 negative for cercariae
 - 18 with monostome cercariae
 - 1 with echinostome cercariae
 - 2 with stylet cercariae
- M. exacuus*
 - 3 negative for cercariae
 - 1 with echinosome cercariae
 - 1 with stylet cercariae
 - 1 with monostome cercariae
 - 1 with spirorchid cercariae
- Physa* sp.
 - 1 large specimen negative for cercariae

The rest of the snails in this experiment were examined a week later on June 14, 1940, with the following results:

- G. parvus*
 - 8 negative for cercariae
 - 7 with monostome cercariae
- M. exacuus*
 - 3 negative for cercariae
- Physa* sp.
 - 1 large specimen negative for cercariae
 - 4 small specimens negative for cercariae
 - 2 small specimens with *C. gyrauli*

Controls for this experiment were collected on June 15, 1940, from precisely the same spot from which the experimental snails were col-

lected. One hundred and twenty-five *Physa* sp. of unquestionably the same generation as those used in the experiment were examined by removing the snail body from the shell and teasing it apart under a dissecting microscope. None of these snails was found to harbor *C. gyrauli* or any other cercaria that might be confused with it. Since two out of the six physas in the experiment and none of the 125 controls were infected with *C. gyrauli* it seems reasonably safe to assume that the cercariae in the experimental snails resulted from infections with the miracidia from the blackbird schistosome. These two infections were just reaching maturity which means that it required an interval of four to five weeks from the time the miracidia penetrated the snail host until cercariae were fully developed. This interval is in accordance with that for the development of *C. elvae* as shown by an earlier experiment (Brackett, 1940c).

The relationship of this schistosome to its snail intermediate host is confusing but very interesting. Since *C. gyrauli* was described from the snail *G. parvus* but was not found in physas from the same locality (Brackett, 1940b), it seems strange that in the above experiment this species should have developed in the physas and not in *G. parvus*. In view of Talbot's (1936) work in which he showed that three species of cercariae very similar in morphology developed in different snail hosts it might be suspected that we are dealing here with a species separate from but similar to *C. gyrauli* and one which requires a different snail host. Looking at Talbot's work further it is seen that in addition to having different hosts these three species (*C. elvae*, *C. stagnicolae*, and *C. physellae*), while almost indistinguishable morphologically, do differ quite strikingly in their behavior. Such is not the case with *C. gyrauli* and the cercariae developed in the above experiment for these latter cercariae agree perfectly in behavior with those which were used by the author (Brackett, 1940b) to establish the species *C. gyrauli*. A likely explanation is that a species of snail may not be equally susceptible to the invasion of miracidia of *P. gyrauli* at all stages in its developmental cycle. Consequently, it may be assumed that when *C. gyrauli* was found in 1939 in a large percentage of the snail *G. parvus* from Sun Prairie Pond, Wisconsin, the snails of this species were at a different stage in their cycle than those used in the experiment described in this paper. The physas examined from the Sun Prairie Pond in 1939 were not found to harbor *C. gyrauli* so, accordingly, they must have been at a stage in their cycle which was refractory to the miracidia of *G. gyrauli* when these miracidia were present.

There is another possibility that might be worthy of consideration. It was mentioned earlier that the miracidia of *G. gyrauli* are attracted towards light so that in their natural environment they would probably concentrate near the surface of the water. It seems possible that under

the natural conditions at the Sun Prairie Pond during the period these miracidia were in the water the physas might have been deeper in the water while the gyrauli were at the surface. An exact antithesis would then have to be assumed for the snails in the experimental aquarium. No observational records are available on this point.

It is obvious that no proved explanation for this host relationship is available. However, future work on the life cycles of the avian schistosomes may be expedited if this point is kept in mind and the conditions of the experiments broadened sufficiently in order to decrease the possibilities of failing to establish an infection even though the right hosts are being used. More extensive work on the life cycle of the blackbird schistosome would surely clarify the conditions necessary for successful establishment of its larval stages in the snail host.

If it is proved that the snail intermediate host is not equally susceptible to infection with schistosomes at all stages of its development, then the epidemiology of schistosome dermatitis becomes more complicated. It has been pointed out several times (Cort et al, 1940 and Brackett, 1941) that varieties of the snail *S. emarginata* are most important in outbreaks of swimmer's itch because they prefer sandy beaches and reach the height of their development during the swimming season. Also of significance is the fact that the dermatitis-producing cercaria *C. stagnicolae* in these snails reaches the peak of its development and emerges in greatest numbers during the height of the resorting season in Michigan and vicinity. It has been suggested (Cort et al, 1940) that the seasonal periodicity of this cercaria is due to the activity of the as yet unknown definitive host. For example, if the definitive host is a migratory bird it would be present on the beaches of northern Michigan only certain times of the year thus limiting the exposure of snails to an infection. If in addition to this we must consider the differential seasonal susceptibility of the snail intermediate host then the interrelationship between the factors influencing or favoring the completion of the life cycles of certain schistosomes is more complex and precise than had been suspected.

DISTRIBUTION OF *Gigantobilharzia gyrauli*

The adults of *G. gyrauli* were first found at Sun Prairie Pond, Wisconsin, in the spring of 1940. Earlier investigations showed three species of schistosome cercariae, *C. physellae*, *C. elongata*, and *C. gyrauli*, were to be found there but due to restricted collecting privileges only two species of adults were found. The author (Brackett, 1940c) gave evidence to suggest that *P. querquedulae*, which occurs commonly in the teal ducks from the Sun Prairie Pond, is the adult stage of *C. physellae* and concluded that the undescribed schistosome found in pied-billed grebes from this pond must be the adult stage of either *C. elongata* or *C. gyrauli*.

Since *C. gyrauli* has been shown to be the larva of *G. gyrauli* in blackbirds, then it seems possible that the grebe schistosome develops from *C. elongata*.

After finding that a large percentage of the blackbirds from the Sun Prairie Pond were infected with *G. gyrauli* (60 per cent of 7 red-winged blackbirds and 83 per cent of 6 yellow-headed blackbirds), these species from fourteen other localities were examined. This schistosome was found to be widely but unevenly distributed in central Wisconsin. Where it occurred it was found in a large percentage of the blackbirds but in other similar and often nearby localities it was completely absent (see Table 1).

TABLE 1.—Blackbirds* examined for *P. gyrauli* in 1940

Locality	Date	Number examined	Number positive	Per cent positive
Sun Prairie	May			
	6-8	7	4	69
	4-8	6	5	83
		(yellow-headed)		
Lake Monona	7-30	8	0	...
Dunn's marsh	7	4	0	...
Hammersley's marsh	16	5	0	...
Hope Lake	15-29	6	5	83
Red Cedar Lake	29	3	2	66
Sumner township Jefferson Co. .	9	6	2	33
			(light inf.)	
Koshkonong station	9	3	0	...
Lake Poygan	13	3	2	66
Winneconne	12	2	2	100
Lake Butte des Morte	13	2	2	100
Rush Lake	11	5	1	20
			(light inf.)	
		5	0	...
		(yellow-headed)		
Horicon marsh	21	5	0	...
Hustiford Lake	22	2	0	...

* Unless otherwise indicated these are all red-winged blackbirds.

These irregularities in distribution do not seem to be due to chance but emphasize a fact which is already known to parasitologists and which was referred to earlier in this paper, namely, that the very special combination of factors permitting the completion of a trematode life cycle is not uniformly distributed. Those blackbirds examined early in the summer were probably carrying infections contracted the previous year because examinations of snails from the same areas during the spring up to the time the birds were examined showed that the cercaria of *G. gyrauli* was not yet escaping into the water. It is evident then that the blackbirds must have returned to the same place they had spent the previous year otherwise the distribution of the parasite would have been more uniform throughout the central Wisconsin marsh and lake region. The persistence with which many species of birds return to the same spot each year has been clearly shown by the banding records of the U. S. Biological Survey, but it seems of considerable interest and perhaps may prove to be

of some significance that such a definite relationship can be shown between a birds migratory habits and its parasitic infections.

BIBLIOGRAPHY

- BRACKETT, STERLING 1940a Studies on schistosome dermatitis. V. Prevalence in Wisconsin. *Am. J. Hyg.* 31 (Sect. D): 49-63.
- 1940b Two new species of schistosome cercariae from Wisconsin. *J. Parasitol.* 26: 195-200.
- 1940c Studies on schistosome dermatitis. VIII. Notes on the biology of the snail hosts of schistosome cercariae in Wisconsin and epidemiological evidence for the life cycles of some avian schistosomes. *Am. J. Hyg.* 32 (Sect. D): 85-104.
- 1941 Schistosome dermatitis and its distribution. Symposium on Hydrobiology. Univ. Wis. Press.
- CORT, W. W., D. B. McMULLEN, LOUIS OLIVIER, AND STERLING BRACKETT 1940 Studies on schistosome dermatitis. VII. Seasonal incidence of *Cercaria stagnicola* Talbot, 1936, in relation to the life cycle of its snail host *Stagnicola emarginata angulata* (Sowerby). *Am. J. Hyg.* 32 (Sect. D): 33-69.
- EJSMONT, L. 1929 Über zwei Schistosomatidengattungen der Vögel. *Bull. Internat. Acad. Polon. Sc. et Lett., Cracovie, Cl. Sc. Math. et Nat., Ser. B*: 389-403.
- LAL, M. B. 1937 Studies on the trematode parasites of birds. II. Morphology and systematic position of some new blood flukes of the family Schistosomatidae. *Proc. Indian Acad. Sc.* 6 (Sect. B): 274-283.
- MCLEOD, J. A. 1937 Two new schistosomid trematodes from water-birds. *J. Parasitol.* 23: 456-466.
- 1940 Studies on cercarial dermatitis and the trematode family Schistosomatidae in Manitoba. *Canad. J. Res. D*, 18: 1-28.
- MILLER, JR., H. M. 1923 Notes on some furcocercous larval trematodes. *J. Parasitol.* 10: 35-46.
- ODHNER, T. 1910 *Gigantobilharzia acotylea* n. g., n. sp. ein mit den Bilharzien verwandter Blutparasit von enormer Länge. *Zool. Anz.* 35: 380-385.
- OISO, T. 1927 On a new species of avian Schistosoma developing in the portal vein of the duck, and investigations of its life history. Taiwan Igakkwai Zasshi, Sept.
- SZIDAT, L. 1930 *Gigantobilharzia monocotylea* n. sp., ein neuer blutparasit aus Ostpreussischen Wasservögeln. *Z. Parasitenk.* 2: 583-588.
- 1939 *Pseudobilharziella filiformis* n. sp., eine neue Vogelbilharzie aus dem Häckerschwann *Cygnus olor* L. *Z. Parasitenk.* 10: 535-544.
- TALBOT, S. B. 1936 Studies on schistosome dermatitis. II. Morphological and life history studies on three dermatitis-producing schistosome cercariae, *C. elvae* Miller, 1923, *C. stagnicola* n. sp., and *C. physellae* n. sp. *Am. J. Hyg.* 23: 372-384.

EXPLANATION OF PLATES

All figures semidiagrammatic

PLATE I

- FIG. 1. *Pseudobilharziella waubensis*. Anterior portion of female.
FIG. 2. *Pseudobilharziella waubensis*. Anterior portion of male.
FIG. 3. *Pseudobilharziella kegonsensis*. Anterior portion of male.
FIG. 4. *Pseudobilharziella horiconensis*. a. Anterior portion of male. b. Posterior portion of male.
FIG. 5. *Pseudobilharziella burnetti*. Anterior portion of male.

PLATE II

- FIG. 6. *Gigantobilharzia gyrauli*. Anterior portion of female.
FIG. 7. *Gigantobilharzia gyrauli*. Anterior portion of male.
FIG. 8. *Gigantobilharzia lawayi*. Anterior portion of female.
FIG. 9. *Gigantobilharzia lawayi*. Anterior portion of male.

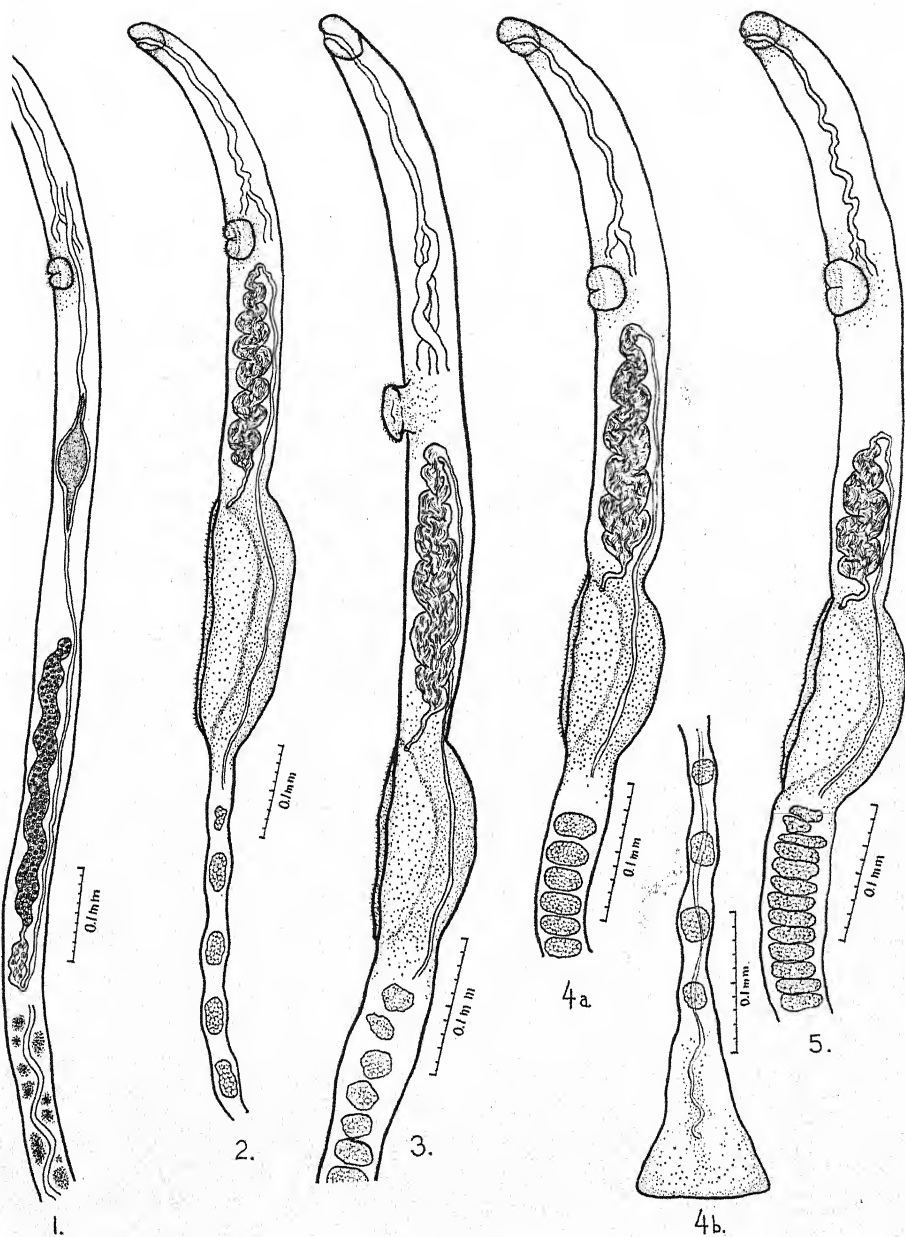


PLATE I

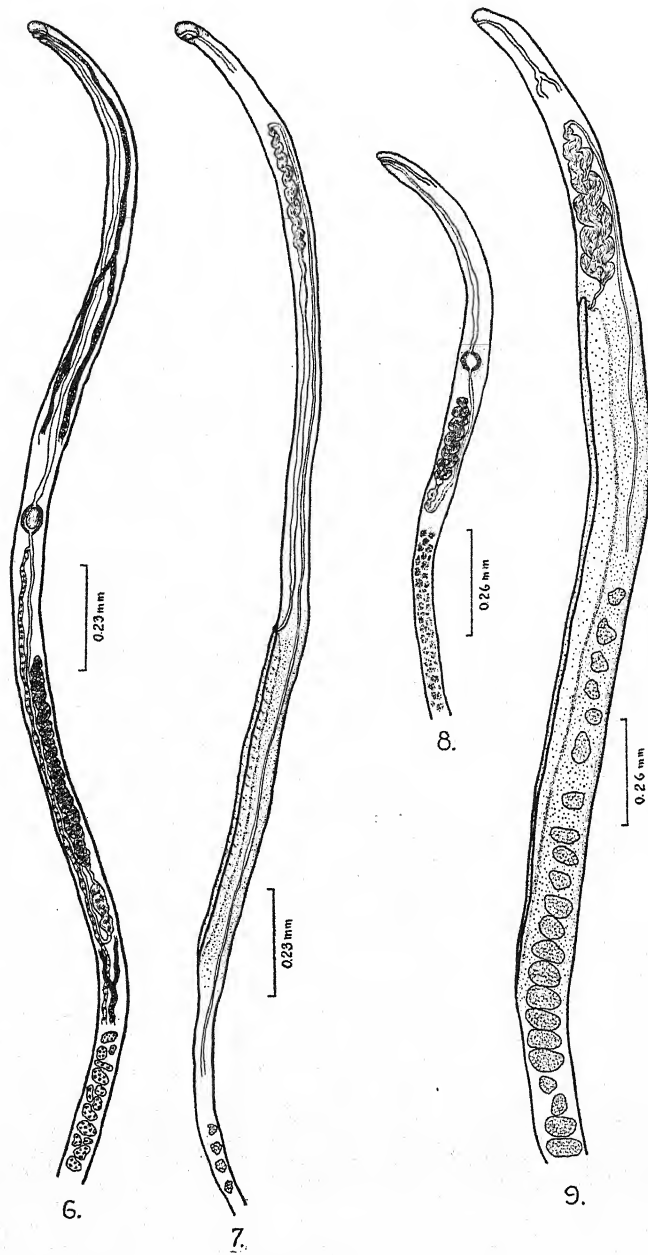


PLATE II

REACTIONS OF MAN TO THE FEEDING OF REDUVIID BUGS

SHERWIN F. WOOD

Department of Life Sciences, Los Angeles City College,
Los Angeles, California

In reports of Kofoid and Whitaker (1936), Herms (1939), Wehrle (1939), and Wood (1941), observations have been recorded concerning the reactions of man after being bitten by various species of *Triatoma* (Hemiptera, Reduviidae). These reactions vary from a slight local redness at sight of bite to a severe illness lasting several days or weeks. In order to record the physical effects of the bite, the writer tested the feeding reactions of several species of uninfected bugs. The bug was allowed to feed on the left hand or arm while using the right hand to record observations.

OBSERVATIONS

On August 3, 1939, an adult female *Triatoma protracta* (Uhler) was placed upon a black-topped table beside my hand. The bug immediately extended its proboscis and began to probe the inside surface of the base of the little finger. A slight pricking sensation was felt as the tip of the proboscis was pressed against the skin and feeding started. After half a minute, the bug moved its body slightly and a more distinct pricking sensation was felt for 3 seconds. The antennae were held outward and slightly extended backwards while the body position was maintained by slight movements of the legs to adjust for slight movements of my hand. After 5 minutes, the bug's body could be seen to swell. After 6 minutes, the tips of the wings began rising above the noticeably rounded abdomen. Twelve and one-half minutes after contact, the bug withdrew its proboscis, having fed to capacity. Careful scrutiny of the finger at the exact point of contact did not reveal any evidence of a puncture, of swelling or redness. Repeated examinations failed to reveal any physical signs of a *Triatoma* having fed upon the finger.

Mr. "S" from Sanger, Fresno County, California, sent me one adult female *Triatoma protracta* with the following note, dated May 21, 1940: "This bug bit me on the hand last night. It evidently struck a vein for in ten minutes I was itching to my ankles and pains were shooting to my shoulder. This morning my hand is badly swollen. As yet the mark of the bite does not show but will when the swelling goes down. They affect my wife differently. She will not feel the bite usually but the next day will have a bad headache. She has learned to recognize the head-

Received for publication, February 22, 1941.

aches and when she has one we look and usually find the bug full of blood. I have known people in the neighborhood to become unconscious from the bites but thought it might be hysterics."

On July 8, 1939, an adult female *Triatoma protracta woodi* Usinger was placed beside my left hand which was palm down on the table. The bug extended its proboscis and immediately began probing. After trying several spots, it wandered away. No definite sensation could be felt as it moved the tip of its proboscis here and there over the finger. Turning my hand over, the bug was placed beside the little finger. The same probing technique was repeated, the bug seeming to avoid calloused areas of the finger. Soon its proboscis came to rest in a crease near the base of the little finger. Almost immediately, its body began to swell and after 5 minutes it withdrew its proboscis. At the site of puncture, there was a slightly reddened area 1 mm in diameter which soon disappeared so that a few minutes later no trace of the point of contact could be found. No pricking sensation of any kind was definitely felt at this feeding. On March 2, 1940, another adult female *T. p. woodi* fed on the inner surface of my left wrist. As the proboscis touched the skin and the bug pressed toward the wrist, a slight tickling sensation was felt for 1 minute. The bug began to feed immediately and no sensation of any kind could be felt indicating its presence. Eight minutes after contact a sensation was felt at the site of contact with the proboscis for a few seconds as if the skin had been touched lightly with a blunt, cold dissecting needle. Eleven and one-half minutes after contact the bug withdrew its proboscis, filled to capacity.

On September 10, 1939, an adult male *Triatoma longipes* Barber was placed on the table. The bug walked about for a few minutes then began slowly to wave its antennae back and forth as if sensing my warm hand. Very soon it extended its proboscis and touched the calloused under-surface of the thumb resting on the table. A slight but definite sensation of touch was felt as the proboscis contacted the thumb. Two and one-half minutes later a distinct tickling sensation was felt for 1 second (movement of the stylets in the epidermis?). Five minutes after contact a distinct tickling sensation was felt for 3 seconds. Six minutes after contact, the upper surface of the abdomen of the bug began to elevate, lifting the wing tips off the abdomen. At 6.5 minutes after contact, the bug withdrew its proboscis and walked away, being only about half full. No physical evidence of the contact could be seen.

On October 21, 1939, my left index finger was suspended in a jar containing 1 male and 1 female *Triatoma heidemanni* Neiva. Both bugs immediately reacted to the warmth by probing the tip of the finger. In 1 minute, both had made contact and started to feed. A definite sensation of something touching the tip of the finger was noticed, followed by a

slight tickling sensation for about 1 minute. One minute after contact, the abdomens of both bugs began to swell. Two and one-fourth minutes after contact, the wing tips of both were well raised above the ends of their abdomens. Three and one-half minutes after contact, a slight tickling sensation lasting several seconds was felt. Both bugs remained practically motionless during feeding. After $4\frac{1}{4}$ minutes, both bugs broke contact. No evidence of any physical damage to the finger at points of contact with the proboscides of the bugs could be found.

On May 18, 1940, an adult male *Triatoma gerstaeckeri* (Stål) made contact with the tip of my left index finger suspended in a glass jar. As it started to feed a sharp pricking sensation was noticed for about 1 minute (movement of stylets, ejection of saliva?). Thereafter, no sensitivity to the presence of the proboscis was noted. The only movements of the bug were slow waving of the antennae and movement of the legs to adjust body position for maintaining contact with slight movements of the finger. After $15\frac{1}{2}$ minutes, the bug broke contact, having fed to capacity.

On July 13, 1940, an adult female *Triatoma sanguisuga* Lec. after 15 minutes of probing, retreating, testing and retesting the tip of the finger (as well as the surface of the nail) suspended in its jar, finally began to feed. Although there were many contacts of the tip of the bug's proboscis with the top of the finger, no sensation of any kind was felt until the proboscis stopped and the bug pressed the tip against the finger. This was followed by a slight tickling sensation for 3 seconds. The bug then remained perfectly motionless, the tip segment of its proboscis at right angles to the skin surface and the rest of the proboscis held at about a 45 degree angle. After 10 minutes, it slowly moved its antennae. Its abdomen was well rounded with the ends of the wings extending well above the top of the abdomen. After 23 minutes the bug seemed full but still held contact. After 29 minutes the finger was moved but the bug moved its body to maintain contact. At 30 minutes movements of the finger resulted in the bug pulling away. By this time, the abdomen of the bug was almost round in cross section like that of large nymphs after a full meal. On August 5, 1940, this same bug was placed on the black table top beside my forearm. Contact with my skin was made on the arm just below the elbow. There was no sensation of any kind as it began to feed. Five minutes later, the wing tips began to lift above the end of the abdomen. After 6 minutes, a tickling sensation was felt for 3 seconds. After 7 minutes, a slight "burning" sensation at the site of the bite was felt for 20 seconds. Eleven minutes after contact, a slight stinging sensation at the point of contact with the proboscis was felt for 15 seconds. After 12 minutes, the bug seemed filled to capacity but continued contact. Twenty-two minutes after contact, the external genitalia,

ordinarily not conspicuous, were forced to protrude by the pressure of the food on the abdominal organs. The conjunctivae between the abdominal segments were distended so that there were broad alternating light and dark bands across the posterior half of the abdomen. The bug pulled away from the arm of its own accord after 24 minutes of feeding. No physical reaction of any kind was found at the site of the bite and there was no feeling of discomfort during the feeding period or thereafter.

On July 23, 1940, an adult female *Triatoma rubida* (Uhler) began to feed almost as soon as my middle finger was suspended in its jar. The proboscis came to rest on the soft skin on top of the terminal joint. A slight pricking was felt for one-half minute, accompanied by a sensation of something touching the finger. The axis of the bug's body was at right angles to the axis of the finger. Its proboscis was held straight out except the tip, which tilted upward. Three and one-half minutes after contact the wing edges began to rise from the posterior edge of the abdomen. After 5 minutes, the abdomen was well rounded. The bug remained very quiet. After 10 minutes, it moved its hind legs as if to get a better stance. At 11½ minutes after contact, it pulled away. The point of contact was in a crease and showed a red spot of clotted blood 0.5 mm in diameter where the wound bled during or after contact with the bug's proboscis. For an area 1 mm in diameter around this spot the skin was slightly reddened. Rubbing the finger removed the clot but the small reddened zone, not swollen, remained for about 1½ hours. Another female *T. rubida* was placed on the table where it fed on the inside of my wrist. As it contacted the skin a definite pricking sensation was felt for 40 seconds followed by a slight tickling at the point of contact. The pricking was felt as the bug pushed its proboscis against the skin. When it stopped pushing, after contact was made, the pricking sensation stopped. When the arm moved, the bug adjusted its position by moving its body on its legs, rarely changing the position of its feet. Eight minutes after contact the bug pulled away, fed to capacity. There was a reddened zone 1 mm in diameter on the skin at the point of contact.

Five female *T. rubida* (2 infected with *Trypanosoma cruzi* Chagas) and one male *T. gerstaeckeri* were received from Mr. "R" who collected them from a house in Sanderson, Texas. Mr. "R" wrote me the following June 27, 1940: "The people living at this place are all in excellent health, and state that these suckers have sure dealt them misery."

On July 23, 1940, my left index and middle fingers were suspended in a jar with two female *Triatoma indictiva* Neiva. Both bugs scurried about the jar and tried to hide. After five minutes, one female moved up to a finger and then backed away without elevating her proboscis. Neither bug seemed inclined to feed. On July 26, the process was repeated for 11 minutes with no results. On August 1, attempts to feed

them in the daytime as above met with failure. This time the females did elevate their proboscides and probed the tip of a finger. They were then placed alongside the inner surface of the forearm and again one female probed several spots on the arm without making contact to feed. No sensation of any kind was felt as the bug moved its proboscis over the surface of the arm. Believing the insects might feed if it were night, I placed my index finger in their jar at 9:55 P.M. The jar was shaded from dim light so that the bugs were barely visible. Five minutes later, one female touched the tip of my finger with her proboscis and remained there for 20 seconds. The other female probed nearby, touching a finger for 30 seconds. Both broke contact and then tried to feed again at the edge of the nail. Two minutes later, as one female probed again, a slight tickling sensation was felt for 2 seconds but in a minute the bug pulled away, retreating into a fold of the paper toweling in the jar. Although a choice of fingers was offered, they did not feed. On August 3, similar results were experienced with the same two female *T. indictiva*. On August 7, noting that the bugs seemed weak, they were placed with a white mouse but neither one fed.

On August 1, 1940, one adult male *Paratriatoma hirsuta* Barber was placed on a pleated piece of paper toweling near the inside of my left wrist. The bug immediately extended its proboscis, inserting it on the undersurface of the wrist. A sharp pricking sensation was felt for 4 seconds, followed by a slight tickling sensation for 5 seconds. One and one-half minutes after contact, the abdomen of the bug began to swell, elevating the wings off the abdomen. By three minutes, the abdomen was markedly distended so that the upper sides showed the red color of the blood in the internal organs beneath. Six minutes after contact, there was a sharp pricking sensation for about 2 seconds. The bug stopped feeding in 6½ minutes, filled to capacity. Another adult male *P. hirsuta* began feeding almost immediately. With the first contact on the skin, there was a brief sharp pricking sensation lasting 10 seconds. The bug immediately began to fill up. Two minutes after contact, the abdomen could be seen enlarging, causing the ends of the wings to be raised. Five minutes after contact, a sensation of something touching lightly at the point of contact was felt for 5 seconds. This bug fed to capacity in 9½ minutes and pulled away. In both cases, no physical disturbance to my skin of any kind could be found at the time of feeding. Forty hours after the bug stopped feeding, a severe itching of the underside of the left wrist directed my attention to the two sites where *Paratriatoma* had fed. The area around each bite was swollen and inflamed. A slight, continuous itching sensation of these areas was felt from forty hours on. Fifty-two hours after the bug had stopped feeding, the round, swollen, reddened areas measured 4 mm in diameter and the itching was less intense. At 70

hours, the "bumps" measured 3 mm in diameter, were still firm but only slightly inflamed. At 94 hours, at one site a hard lump, 3 mm in diameter, was evident. At the other site, there was only a slight elevation and the area affected measured 2 mm in diameter. At 118 hours, both elevated areas measured 2 mm across and appeared bluish red in contrast to the former pinkish red. The swelling at one site had disappeared but the other site was still firm and hard although somewhat smaller than at the previous observation. Seven days after being bitten, there were two purplish-red areas about 2 mm in diameter with only the central point raised but no physical sign of an actual puncture mark could be found. At 15 days, two discolored areas at the site of the bites were distinctly visible. They measured 2 mm in diameter and were gradually fading, especially at the edges. At 17 days, the one bite which had remained swollen and hard longer than the other was now barely visible. The other site was a deeper red in color and showed a hard, swollen, centrally elevated region 1 mm in diameter after having shown no swelling for several days. At 19 days, there was no trace of the one bite. The other bite showed a swollen, reddened, elevated area 2 mm in diameter which was recognizable only as a tiny pink dot at 30 days.

DISCUSSION

Since it has been shown that these bugs are vectors of Chagas' disease (F. D. Wood, 1934; Kofoid and Whitaker, 1936; Packchanian, 1939; S. F. Wood, 1941) in the United States, distinction should be made between symptoms which are purely reactions to physical and chemical (salivary secretions) contacts with the bugs and possible reactions to disease organisms that they may carry. The method of transmission of *Trypanosoma cruzi*, causative agent of Chagas' disease, is by contamination of the bug bite, of a skin abrasion or of a mucous membrane by infective feces of the bug. As has been emphasized recently by Mazza (1939), a very prevalent and easily recognizable symptom of Chagas' disease is the swelling of one or both eyelids (unilateral or bilateral palpebral edema) after contact with infected insects.

SUMMARY

The writer experienced no marked physical discomfort during feedings of *Triatoma protracta*, *T. p. woodi*, *T. longipes*, *T. heidemannii*, *T. gerstaeckeri*, *T. sanguisuga*, *T. rubida*, and *Paratriatoma hirsuta* at different times upon various areas of his left fingers, hand and forearm. In every case but one, there was no marked injury due to the bite. In the case of *Paratriatoma hirsuta* Barber, there was a local irritation of the skin at the site of the bites, but there was no general reaction of any kind.

BIBLIOGRAPHY

- HERMS, W. B. 1939 Medical Entomology. New York, Macmillan, 3rd Ed. 582 pp.
- KOFOID, C. A. AND WHITAKER, B. G. 1936 Natural infection of American human trypanosomiasis in two species of cone-nosed bugs, *Triatoma protracta* Uhler and *Triatoma uhleri* Neiva, in the western United States. J. Parasitol. 22: 259-263.
- MAZZA, S. 1939 Inexistencia de un síntoma patognomónico en formas agudas de enfermedad de Chagas. Prensa Méd. Argent. 26: 1-32.
- PACKCHANIAN, A. 1939 Natural infection of *Triatoma gerstaeckeri* with *Trypanosoma cruzi* in Texas. Pub. Health Rep., U. S. Pub. Health Serv. 54: 1547-1554.
- WEHRLE, L. P. 1939 Observations on three species of *Triatoma* (Hemiptera: Reduviidae). Bull. Brooklyn Entom. Soc. 34: 145-154.
- WOOD, F. D. 1934 Natural and experimental infection of *Triatoma protracta* Uhler and mammals in California with American human trypanosomiasis. Am. J. Trop. Med. 14: 497-512.
- WOOD, S. F. 1941 New localities for *Trypanosoma cruzi* Chagas in southwestern United States. Am. J. Hyg. 34: 1-13.

STRIGEID TREMATODES OF THE ALLIGATOR, WITH REMARKS ON THE PROSTATE GLAND AND TERMINAL PORTIONS OF THE GENITAL DUCTS

ELON E. BYRD AND ROBERT J. REIBER

Zoology Department, University of Georgia

Within recent years at least five species of strigeid trematodes have been made available for study by the writers. These worms were recovered from the small intestine of the alligator, *Alligator mississippiensis* (Daudin), from three localities, Silver Springs, Florida, Tallahassee, Florida, and Midville, Georgia. Since all five species of the flukes possess a prostate gland, they have been placed in the family of PROTERODIPLOSTOMIDAE Dubois, 1936. Three of the species are considered new to science and two of these are placed in a new genus. A description of the new genus and species follows.

Family PROTERODIPLOSTOMIDAE Dubois, 1936

Subfamily PROTERODIPLOSTOMINAE Dubois, 1936

Pseudoneodiplostomum acetabulata n. sp.

(Figs. 1-2)

Specific diagnosis: Pseudoneodiplostomum. Elongated strigeid fluke with body divided into two segments, anterior segment of which is relatively thin and flat; posterior segment almost cylindrical. Total length of body $5.50 \pm .01$ (4.80-5.96)* mm. Anterior segment broadest in region of holdfast organ, tapering anteriorly to a much narrower and more rounded anterior margin, $2.78 \pm .005$ (2.44-2.96) mm long by $1.02 \pm .004$ (0.83-1.22) mm in greatest width. Posterior segment slightly overlapped by anterior segment, attached to anterior segment by antero-ventral margin, widest anteriorly, with almost parallel sides but tapering slightly toward posterior end, $2.74 \pm .006$ (2.24-3.07) mm long by $534 \pm .09$ (320-770) μ in greatest width. Oral sucker muscular, from 130 to 160 μ in diameter, at anterior margin of body. Prepharynx short or absent. Pharynx 87 to 110 μ in diameter. Esophagus short, about same length as pharynx. Ceca slender tubes, curving around acetabulum and holdfast organ, reaching to about level of middle of prostate in hindbody. Acetabulum large and muscular, $410 \pm .01$ (370-450) μ in diameter, situated $0.96 \pm .003$ (0.77-1.06) mm behind anterior margin of body. Holdfast organ broadly elliptical, muscular, with about 12 papillae bordering central cavity, $680 \pm .002$ (570-840) μ long by $610 \pm .002$ (500-740) μ wide, located $470 \pm .004$ (410-510) μ behind acetabulum, just in front of hindbody. Proteolytic gland large, multi-lobed, dorsal to posterior margin of holdfast organ.

Ovary dorsal in position, to left of midline, $127 \pm .005$ (87-168) μ long by $173 \pm .02$ (110-306) μ wide, placed $0.62 \pm .006$ (0.27-1.13) mm behind union of two body segments. Oviduct long, giving rise to Laurer's canal about midway between ovary and oötype, forming oötype in intertesticular space. Shell gland and yolk reservoir rather large, between the testes. Uterus forming several transverse loops in as-

Received for publication, March 6, 1941.

* Measurements are presented in two ways. 1. The mean with its probable error. 2. The range of measurements (from smallest to largest) in parentheses. In computing the probable error the number of degrees of freedom is taken into account.

cending to anterior limits of hindbody, then forming more transverse loops in descending to genital pore, surrounded by muscle in area of prostate to form metraterm. Metraterm about as long as prostate. Ova 87 to 97 μ long by 44 to 61 μ wide, several dozen usually present in uterus. Vitellaria follicular, distributed into both body segments, extending as far forward as caudal boundary of acetabulum in anterior segment and posteriorly to level of prostate in hindbody; vitellaria in posterior segment in two main bands, dorsal, lateral and ventral to ceca behind ovary but may become continuous band across dorsum of body in anterior part of hindbody.

Testes in tandem, close behind ovary, triangular to rectangular (seldom round) in shape, in middle of hindbody. Anterior testis $220 \pm .01$ (170–330) μ long by $350 \pm .003$ (240–500) μ wide. Posterior testis $213 \pm .02$ (190–339) μ long by $338 \pm .01$ (200–504) μ wide, situated $180 \pm .02$ (84–300) μ behind anterior testis. Vasa efferentia uniting in dorsal area of intertesticular space to form small seminal reservoir. Vas deferens arising from reservoir, passing posteriorly almost parallel to uterus in ventral aspect of body, gradually increasing in width, becoming dilated to form seminal vesicle in area immediately behind testes. Seminal vesicle considerably coiled, constricting into ejaculatory duct near anterior limit of prostate. Ejaculatory duct with thin, muscular wall in central part of its length, joining prostatic canal at level approximating one-fourth length of prostate from caudal end. Prostate rather large, 310 to 450 μ long by 120 to 160 μ wide, with heavy muscular wall and medium-large central cavity. Prostatic canal joined by uterus at end of prostate, forming hermaphroditic canal. Hermaphroditic canal opening at summit of genital papilla into rather large atrium. Genital atrium dorsal in position. Prostate gland, ejaculatory duct (muscular portion) and metraterm surrounded by their own individual masses of gland cells; prostate surrounded by more of these cells than is the case with other two structures.

Host: *Alligator mississippiensis* (Daudin).

Habitat: Small intestine.

Locality: Silver Springs, Florida.

Additional specimens: Tallahassee, Florida, and Midville, Georgia.

Type specimen: U. S. Nat. Mus. Helm. Coll. No. 44968. *Paratype:* 44969.

The present species is considered distinct from the other two species, *Pseudoneodiplostomum siamense* (Poirier) and *P. thomasi* (Dollfus), now assigned to the genus, due to its larger body size, the more posterior distribution of the vitellaria in the hindbody and the much larger acetabulum. More specifically, *P. acetabulata* can be separated from *P. thomasi* by its larger body size, the more elongated hindbody, the more posterior position of the gonads, the larger ventral sucker and the greater posterior extension of the vitellaria in the hindbody. From *P. siamense* the present species differs in regards to the larger body size, the much larger ventral sucker, the more posterior distribution of the vitellaria, the absence of large, flaring, finger-like papillae about the margin of the holdfast and in the nature of the prostate.

Subfamily POLYCOTYLINAE Monticelli, 1888

Polycotyle ornata Willemoes-Suhm, 1870

(Figs. 3–4)

Specific diagnosis: *Polycotyle*. Small to medium-sized, bisegmented strigeid fluke with slight dorsal curvature of posterior segment, $2.30 \pm .01$ (1.81–3.12) mm in length. Anterior segment $1.13 \pm .006$ (0.87–1.48) mm long by $550 \pm .003$ (430–650) μ wide in region of holdfast organ. Posterior segment $1.19 \pm .008$ (0.96–1.60)

mm in length, attached to postero-dorsal margin of anterior segment, partly overlapped by forebody. Oral sucker at anterior margin of body, approximately $35\ \mu$ in diameter. Prepharynx short or absent. Pharynx same size or slightly smaller than oral sucker. Esophagus about three times as long as pharynx. Ceca slender tubes, curving around holdfast organ, reaching to near level of genital papilla. Ventral sucker transversely oval, $75 \pm .004$ (52–87) μ long by $103 \pm .006$ (87–130) μ wide, situated $484 \pm .02$ (390–610) μ behind anterior margin of body. Holdfast organ elongated elliptical, muscular, with about 12 to 15 short papillae bordering central cavity, $243 \pm .02$ (190–350) μ long by $183 \pm .01$ (174–260) μ wide, situated $90 \pm .01$ (61–174) μ behind acetabulum and $169 \pm .01$ (130–240) μ in advance of caudal margin of forebody. Proteolytic gland conspicuous, median in position, dorsal to caudal margin of holdfast organ. Nerve ring and lateral nerve trunks visible in anterior part of forebody.

Ovary transversely oval, $98 \pm .006$ (87–130) μ long by $126 \pm .006$ (100–150) μ wide, located slightly to right of midline at caudal margin of forebody, from 43 to 240 μ in front of testes. Shell gland immediately behind ovary and slightly more to right in position. Yolk reservoir large, to left of position of shell gland, immediately behind ovary. Oviduct short, giving rise to Laurer's canal at about level of caudal boundary of ovary, forming oötype in space between ovary and testes. Uterus slightly convoluted, ascending to extreme anterior margin of hindbody before descending to genital pore, with small sphincter muscle immediately in front of its union with the ejaculatory duct, containing up to 10 ova. Ova 87 to 96 μ in length. Vitellaria follicular, confined to anterior segment, from level of acetabulum to caudal boundary of forebody, more concentrated in region of holdfast organ.

Testes posterior to shell gland and yolk reservoir, in tandem or diagonal positions; anterior testis displaced slightly to right of midline and posterior one a little to left of midline, often with levels overlapping somewhat but not infrequently with posterior testis as much as 50 μ behind anterior testis. Anterior testis transversely ovoid to triangular, $154 \pm .01$ (113–240) μ long by $205 \pm .005$ (174–220) μ wide. Posterior testis of same shape as anterior, $146 \pm .01$ (113–191) μ long by $201 \pm .005$ (174–230) μ wide, situated $776 \pm .004$ (620–940) μ in advance of caudal margin of hindbody. Vasa efferentia arising from main body of their respective testis, ascending to space between ovary and testes to form small seminal reservoir. Vas deferens descending from reservoir to space behind caudal testis, forming seminal vesicle. Seminal vesicle greatly convoluted, narrowing into ejaculatory duct which proceeds posteriorly alongside of prostate to discharge into uterus. Canal formed by fusion of uterus and ejaculatory duct short, discharging almost immediately into canal of prostate. Prostate muscular, elongated, approximately 450 μ long by 52 μ in greatest width, surrounded by rather large, evacuated prostatic gland cells. Prostatic canal with corrugated, cuticular lining, joining with canal of uterus and ejaculatory duct to form hermaphroditic canal. Hermaphroditic canal short, opening to outside at summit of the genital papilla into rather large, dorsal atrium. Posterior body segment with median, dorsal row of 17 to 19 suckers, beginning at level of ovary and terminating at atrium. Largest sucker in posterior position of row, 130 μ in diameter; suckers diminish in size toward anterior end of row until smallest measures only 20 μ in diameter. In sectioned material two small suckers may be observed between last two suckers in dorsal row. A single, large sucker, measuring 130 by 170 μ , is placed in the atrium, immediately behind genital papilla.

Host: *Alligator mississippiensis* (Daudin).

Habitat: Small intestine.

Locality: Silver Springs, Florida.

Additional material: Tallahassee, Florida, and Midville, Georgia.

The material collected by us is considered to be identical with that described by Willemoes-Suhm (1870), Poirier (1886), Dubois (1936) and others, although none of the specimens in the present collection are

as large as those considered by these writers. In practically every detail our material is smaller. Only one other significant difference is to be noted; there are at least seventeen suckers in the median, dorsal row on the posterior segment of the body, whereas only fifteen suckers have been reported previously as constituting this row. In some of our specimens we have been able to count as many as twenty of these suckers in living material, although on fixation and mounting it is found that the density of the stain in the ovary and its complex renders the more anterior suckers more difficult to see. In one of the sectioned worms eighteen suckers are present while in another there are nineteen. In the sectioned specimens two very small suckers are demonstrated in the area between the last two suckers in the dorsal row.

Crocodilicola pseudostoma (Willemoes-Suhm, 1870)

(Figs. 5-6)

Specific diagnosis: *Crocodilicola*. Small strigeid fluke with unsegmented body or with body segmentation only feebly indicated in region of ovary where thin, flat anterior segment unites with almost cone-shaped posterior segment, measuring 1.60 to 1.80 mm in length for entire body. Anterior segment rounded to slightly pointed anteriorly, broadening posteriorly to attain maximum width in region of holdfast organ, 1.00 to 1.20 mm long by 560 to 620 μ in width at widest part. Posterior body 710 to 740 μ long by 400 to 460 μ wide, widest in anterior region, tapering to blunt point at posterior end. Oral sucker 43 μ in diameter, located at extreme anterior margin of forebody. Prepharynx short or absent. Pharynx 35 μ in diameter. Esophagus about 70 μ long. Ceca slender tubes, reaching to near genital papilla. Acetabulum transversely oval, 60 to 70 μ long by 113 to 130 μ wide, located 450 to 590 μ behind anterior margin of body. Holdfast organ slightly ovoid, about 200 μ in diameter, situated 190 to 220 μ behind acetabulum and very near caudal boundary of forebody. Nerve ring and lateral nerve trunks visible in forebody to vitellaria.

Ovary transversely oval, 70 μ long by 140 to 160 μ wide, in midline close behind caudal limit of vitellaria. Oviduct long, extending posteriorly, dorsal to anterior testis, to intertesticular space, giving rise to Laurer's canal midway between ovary and oötype. Oötype, shell gland and yolk reservoir between testes. Uterus passing out to right and up to anterior margin of hindbody, swings to left across body to ventral position and downward to genital pore, developing sphincter muscle just before discharging into hermaphroditic canal at end of prostate, containing relatively few ova. Ova 96 to 104 μ long. Vitellaria follicular, confined to forebody, from level of acetabulum to caudal margin of forebody, more concentrated about ceca and holdfast organ. Proteolytic gland conspicuous, at caudal boundary of holdfast organ.

Testes in tandem, transversely oval; anterior testis 130 to 160 μ long by 360 to 370 μ wide, located 30 to 50 μ behind ovary; posterior testis 130 to 174 μ long by 330 to 350 μ wide, separated from anterior testis by shell gland and yolk reservoir, placed 240 to 280 μ in advance of caudal boundary of hindbody. Vasa efferentia arising from opposite sides of their respective testes, passing forward to form small seminal reservoir in space between ovary and testes. Vas deferens arising from seminal reservoir, passing downward in ventral aspect of body, forming greatly dilated seminal vesicle immediately behind caudal testis. Ejaculatory duct arising as narrow tube from seminal vesicle, passing posteriorly between uterus and prostate, joining prostatic canal at about one-third the length of that canal from its posterior end, developing slightly muscular wall just before joining pro-

static canal. Prostate ellipsoidal pouch with comparatively thick, muscular wall and large central cavity. Central cavity with corrugated, cuticular lining. Prostatic gland cells numerous, small, greatly compact in certain areas about prostate. The fused prostatic canal and ejaculatory duct joins uterus at end of prostate to form hermaphroditic canal. This canal is short and opens at summit of short genital papilla into small genital atrium.

Host: *Alligator mississippiensis* (Daudin).

Habitat: Small intestine.

Locality: Silver Springs, Florida.

Although only four mature specimens of this species were recovered from a single alligator, there can be but little doubt that we are dealing with *Crocodylicola pseudostoma*. Two of our mature specimens were used for sectioning and the other two were mounted *in toto*. Our specimens are somewhat smaller in total body size than reported in the literature, but being unable to tell whether or not our specimens are fully matured (of about a dozen specimens collected all but four are considered as immature forms), we prefer to describe them as belonging to the species to which they are assigned. In so far as we can determine our specimens depart from the typical form as described by Dubois (1936b) only in regards to the more transversely oval testes and in the fusion of the ejaculatory duct with the prostatic canal instead of with the uterus.

Pseudocrocodylicola n. g.

Generic diagnosis: POLYCOTYLINAE. Small, bisegmented strigeid flukes with lanceolate anterior segment which is longer than the elongated cylindrical posterior segment. Acetabulum in middle or in advance of middle of anterior segment. Hold-fast organ in anterior limit of last third of anterior segment, with 10 to 12 papillae bordering central cavity. Proteolytic gland present. Vitellaria follicular, confined to anterior segment. Gonads in tandem, in posterior segment; ovary in advance of testes, separated from testes by small seminal reservoir. Shell gland and yolk reservoir between testes. Laurer's canal present. Uterus ascending to anterior limit of posterior segment, descending to genital pore, containing relatively few ova. Seminal vesicle large, between testes and prostate. Ejaculatory duct discharging into prostatic canal. Prostate small, muscular, ellipsoidal in shape, surrounded by relatively few but large gland cells. Uterus and prostatic canal unite to form hermaphroditic canal. Hermaphroditic canal surrounded by a secondary pouch of muscle or a parenchyma of spongy-like tissue containing muscle fibers, dilated into genital cloaca, discharging into small, almost negligible atrium.

Type species: *Pseudocrocodylicola americanense* n. sp.

Additional species: *Pseudocrocodylicola georgiana* n. sp.

Pseudocrocodylicola americanense n. sp.

(Figs. 7-8)

Specific diagnosis: *Pseudocrocodylicola*. Small, lancet-shaped strigeid fluke with bisegmented body, $3.14 \pm .03$ (2.88-3.60) mm in total length. Anterior segment very thin and flat anteriorly, almost parallel sides, slightly overlapping posterior segment ventrally, $2.30 \pm .02$ (2.17-2.44) mm long by $480 \pm .01$ (430-520) μ wide. Posterior segment cylindrical, attached to postero-dorsal surface of forebody, $0.95 \pm .005$ (0.83-1.24) mm long by $250 \pm .01$ (220-270) μ wide. Oral sucker 44 to 52 μ in diameter. Prepharynx short or absent. Pharynx 44 to 61 μ in diameter. Esophagus 130 to 174 μ long. Ceca slender tubes, reaching to level of prostate in

posterior segment. Acetabulum weakly muscular, $100\ \mu$ in diameter, located $1.00 \pm .004$ (0.80–1.20) mm behind anterior margin of forebody. Holdfast organ elliptical, muscular, with about 12 papillae bordering central cavity, $340 \pm .007$ (310–370) μ long by $210 \pm .003$ (190–220) μ wide, located $480 \pm .003$ (310–700) μ behind ventral sucker and $340 \pm .01$ (290–400) μ in advance of caudal margin of forebody. Proteolytic gland small but well developed, dorsal to caudal margin of holdfast organ. Nerve ring and lateral nerve trunks visible in forebody to level of vitellaria.

Ovary 87 to $96\ \mu$ in diameter, to left of midline in anterior part of hindbody. Oviduct descending dorsally to anterior testis, giving rise to oötype in intertesticular space. Laurer's canal present. Shell gland and yolk reservoir between testes. Uterus ascending to anterior extremity of hindbody before descending in ventral aspect of hindbody to genital pore, containing up to 16 ova. Ova 97 to $104\ \mu$ long by 52 to $70\ \mu$ wide. Vitellaria follicular, in forebody from caudal boundary of forebody to or just behind acetabulum.

Testes small, transversely oval, one behind other in anterior two-thirds of hindbody. Anterior testis $180 \pm .004$ (160–220) μ long by $220 \pm .008$ (190–224) μ wide, separated from ovary by small seminal reservoir. Posterior testis $190 \pm .01$ (180–220) μ long by $210 \pm .006$ (170–230) μ wide, from 50 to $100\ \mu$ behind anterior testis. Vasa efferentia arising from opposite sides of their respective testes, passing forward to form small seminal reservoir in space immediately behind ovary. Vas deferens descending from reservoir, gradually increasing in width in descending to seminal vesicle. Seminal vesicle S-shaped, in space behind caudal testis, constricting into ejaculatory duct. Ejaculatory duct passing from left to right side of body, then turning rather sharply inward to join prostatic canal just caudal to middle of prostate. Prostate small, muscular, ellipsoidal pouch, 200 to $300\ \mu$ long by 100 to $150\ \mu$ in greatest width, surrounded by numerous rather large and scattered gland cells. Atrium small. Genital papilla slight elevation into atrium. Genital pore at summit of papilla, passing inward as hermaphroditic canal. Hermaphroditic canal surrounded by muscular pouch that continues along uterus for short distance, becoming dilated at point of union of uterus with prostatic canal. Musculature of prostate penetrates secondary pouch to form a prostatic papilla in dilated part of hermaphroditic canal.

Host: Alligator mississippiensis (Daudin).

Habitat: Small intestine.

Locality: Midville, Georgia.

Type specimen: U. S. Nat. Mus. Helm. Coll. No. 44970. *Paratype:* 44971.

Pseudocrocodilicola georgiana n. sp.

(Figs. 9–10)

Specific diagnosis: *Pseudocrocodilicola*. Small lancet-shaped, bisegmented strigeid fluke, $1.58 \pm .004$ (1.44–1.77) mm in total length. Anterior segment thin and flat anteriorly, widest in region of holdfast, slightly overlapping anterior part of hindbody, $1.08 \pm .004$ (0.96–1.22) mm long by $300 \pm .003$ (280–310) μ in greatest width. Posterior segment cylindrical, attached to posterior-dorsal surface of anterior segment, $610 \pm .003$ (460–810) μ long by $160 \pm .01$ (160–170) μ wide. Oral sucker 35 to $43\ \mu$ in diameter. Prepharynx short or absent. Pharynx same size as oral sucker. Esophagus 70 to $130\ \mu$ long. Ceca narrow tubes, extending to middle of prostate in posterior segment. Acetabulum weakly muscular, $61\ \mu$ in diameter, $520 \pm .002$ (440–570) μ behind anterior margin of forebody. Holdfast organ elliptical, muscular, with about 12 papillae bordering central cavity, $146 \pm .03$ (130–160) μ long by 96 to $100\ \mu$ wide, located $162 \pm .005$ (130–180) μ behind acetabulum and $188 \pm .04$ (170–260) μ in advance of caudal margin of forebody. Proteolytic gland small, dorsal to caudal margin of holdfast organ.

Ovary in anterior part of hindbody, slightly to right of midline, $70 \pm .02$ (61–80) μ in diameter. Oviduct arising from middle of ovary, descending dorsally to anterior testis, giving rise to oötype in space between testes. Laurer's canal

present. Shell gland and yolk reservoir intertesticular. Uterus ascending to anterior limit of hindbody, descending in ventral aspect of body to genital pore, containing up to 11 ova. Ova 70 to 96 μ in length. Vitellaria follicular, confined to forebody, from caudal margin of forebody to level 87 to 174 μ in advance of acetabulum.

Testes in tandem, in anterior three-fifths of hindbody; anterior testis $125 \pm .004$ (113–140) μ long by $138 \pm .02$ (130–150) μ wide, abutting ovary or up to 60 μ behind that organ; posterior testis separated from anterior testis by shell gland and yolk reservoir, $142 \pm .01$ (130–150) μ long by $147 \pm .003$ (130–165) μ wide, located $224 \pm .01$ (174–320) μ from caudal boundary of hindbody. Vasa efferentia uniting in area between ovary and testes, forming small seminal reservoir from which vas deferens arises. Vas deferens descending alongside uterus to area immediately behind testes, forming seminal vesicle. Ejaculatory duct emerging from seminal vesicle to join prostatic canal at anterior end of prostate, entering prostate from antero-ventral aspect. Prostate muscular, ellipsoidal in shape, about 100 μ long, surrounded by relatively few, scattered gland cells. Genital atrium small, with only slight indication of genital papilla. Hermaphroditic canal rather long, greatly dilated, surrounded by parenchyma of spongy-like tissue containing muscle fibers (the secondary pouch), passing inward through secondary pouch from summit of papilla to end of prostate, receiving both the uterus and prostatic canal at that point. Musculature of prostate penetrates secondary pouch.

Host: *Alligator mississippiensis* (Daudin).

Habitat: Small intestine.

Locality: Midville, Georgia.

Type specimen: U. S. Nat. Mus. Helm. Coll. No. 44972. *Paratype:* 44973.

Pseudocrocodilicola georgiana is very similar to *P. americanense* but differs from that species in regards to several important details. It is considerably smaller of body size than *P. americanense*. At first this difference was not considered as important since a goodly number of young adults and immature specimens of the larger species was found in which the body size was identical with that of the more mature specimens of *P. georgiana*. Regardless of body size the relative position of the acetabulum and vitellaria remained constant in each species. In *P. americanense* the vitellaria extends only to the acetabulum while in *P. georgiana* these glands may extend as far as midway between the acetabulum and the anterior margin of the forebody; they never terminate at or behind the acetabulum. It is true that in the larger species the acetabulum may be as much as three times as far away from the holdfast organ as is the case in *P. georgiana*, and this may account for the difference noted in the forward distribution of the vitellaria in the smaller species. However, here again is a constant difference in the specimens of the two species regardless of body size. The only other noteworthy difference between the two species has to do with the terminal parts of the genital ducts. In *P. georgiana* the ejaculatory duct fuses with the prostatic canal at its anterior end; the duct penetrates the prostate at its antero-ventral margin. On the other hand, the ejaculatory duct in *P. americanense* penetrates the prostate and fuses with the prostatic canal posterior to the middle of the prostate.

The genus *Pseudocrocodilicola* is considered to be more closely related to the genus *Crocodilicola* than to the other genera of the subfamily Poly-

COTYLINAE because of the general appearance of the body and its internal organization. However, it is considered to be distinct from that genus because of the more distinct bisegmentation of the body, the more forward position of the holdfast organ, the more cylindrical posterior body segment and the nature of the prostate and associated ducts. Dubois (1936b) described the ejaculatory duct in *Crocodilicola* as entering the uterus rather than the prostatic canal. The duct so formed by these two organs fused with the prostatic canal before they opened into a rather large genital atrium. In our material, identified as *C. pseudostoma*, however, this is not the case, for certainly the ejaculatory duct fuses with the prostatic canal and then this canal is joined by the uterus to form the hermaphroditic canal. Accordingly, no structure comparable with the secondary pouch has been observed in *Crocodilicola*. Such a pouch has developed to very conspicuous proportions in *Pseudocrocodilicola*. In regards to the secondary pouch, we have been unable to find reference to any structure that may be interpreted as being a counterpart of this structure. A cirrus pouch has been described for the species *Heterodiplostomum lanceolatum* by Dubois (1936b), and this bears some resemblance to the secondary pouch of the present genus. In this species, however, the ejaculatory duct does not unite with the prostatic canal as in *Pseudocrocodilicola*, but fuses with the uterus. The hermaphroditic canal so formed discharges into the atrium at the base of the genital papilla while the prostatic canal ramifies the cirrus pouch before opening into the atrium at the summit of the papilla. These two genera further differ in that the vitellaria are distributed into both segments of the body in *Heterodiplostomum*.

DISCUSSION

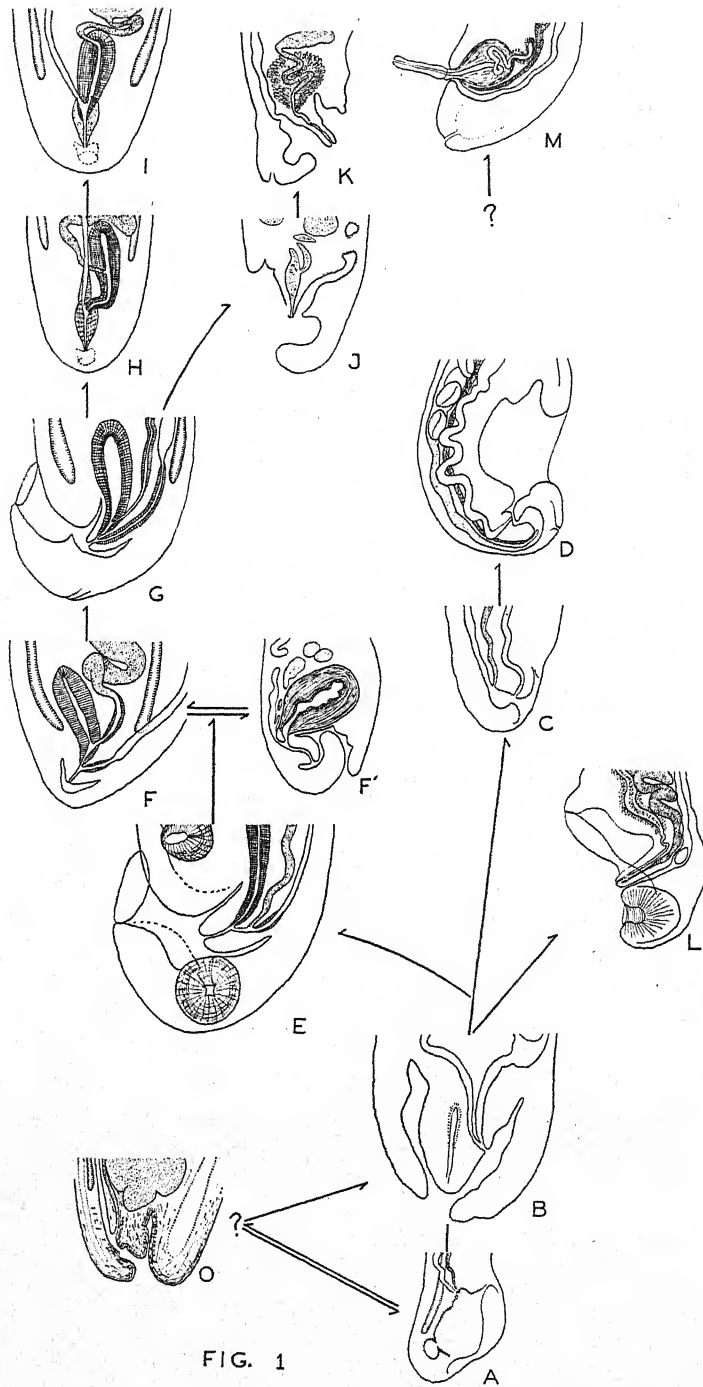
For the strigeid flukes parasitic in the Reptilia, Dubois (1936a) proposed the family PROTERODIPLOSTOMIDAE. Due primarily to differences noted in the size and shape of the holdfast organ (tribocytic organ), the presence or absence of papillae bordering the cavity of the holdfast organ and differences in the distribution of the vitellaria, Dubois recognized two supersubfamilies and three subfamilies in the family group. In the first supersubfamily, PROTERODIPLOSTOMIDI, only those species were included in which the holdfast organ was small to medium-sized and which bore papillae on the margin of its cavity. The supersubfamily was divided into the subfamilies PROTERODIPLOSTOMINAE Dubois, 1936, in which the vitellaria were distributed to both segments of the body, and POLYCOTYLINAE Monticelli, 1888, in which the vitellaria were confined to the anterior segment of the body. The subfamily OPHIODIPLOSTOMINAE Dubois, 1936, in which the holdfast organ is massive and often greatly elongated, and is devoid of papillae, constituted the only subfamily in the supersubfamily OPHIODIPLOSTOMIDI.

The same year Dubois (1936b) diagnosed the family PROTERODIPLOSTOMIDAE to include those strigeid flukes parasitic in reptiles in which the anterior body segment was flat and foliiform, the testes were spherical or ovoid and a prostate, located dorsally to the ejaculatory duct, was present. In the family proper, as now outlined, nine genera were included as follows: 1. *Proterodiplostomum* Dubois, 1936, 2. *Mesodiplostomum* Dubois, 1936, 3. *Prolecithodiplostomum* Dubois, 1936, 4. *Cystodiplostomum* Dubois, 1936, 5. *Herpetodiplostomum* Dubois, 1936, 6. *Paradiplostomum* La Rue, 1926, 7. *Ophiodiplostomum* Dubois, 1936, 8. *Petalodiplostomum* Dubois, 1936, and 9. *Heterodiplostomum* Dubois, 1936. To these nine genera were assigned twelve species, of which all but three were considered as new. In the "addenda" of the family was placed the genera *Polycotyle* Willemoes-Suhm, 1870, *Crocodilicola* Poche, 1925, *Proalarioides* Yamaguti, 1933, and *Pseudoneodiplostomum* Dubois, 1936. These four genera contained five species. In addition to these genera and species, six inadequately described species were placed in the "species inquirendae."

Later the same author (1938b) diagnosed the family PROTERODIPLOSTOMIDAE to include those diplostome parasites of reptiles that are characterized by the presence of a paraprostome, located dorsally to the ejaculatory canal, the efferent canal of which constitutes a third genital canal (exceptions: *Mesodiplostomum gladiolum* Dubois, 1936, and *Proalarioides serpentis* Yamaguti, 1933), and having testes that are spherical or ovoid but never lobed. In the three already mentioned subfamilies the following genera were assigned: 1. *Proterodiplostomum*, 2. *Mesodiplostomum* and 3. *Pseudoneodiplostomum* to the subfamily PROTERODIPLOSTOMINAE; 1. *Polycotyle*, 2. *Crocodilicola*, 3. *Proalarioides*, 4. *Cystodiplostomum*, 5. *Herpetodiplostomum*, 6. *Paradiplostomum* and 7. *Prolecithodiplostomum* to the subfamily POLYCOTYLINAE; and 1. *Ophiodiplostomum*, 2. *Heterodiplostomum* and 3. *Petalodiplostomum* to the subfamily OPHIODIPLOSTOMINAE. The species inquirendae remained about as previously outlined.

To this fairly homogeneous group of strigeid flukes from the Reptilia the present paper adds *Pseudoneodiplostomum acetabulata* n. sp., assigned to the subfamily PROTERODIPLOSTOMINAE, and *Pseudocrocodilicola americanense* n. g., n. sp. and *P. georgiana* n. sp., assigned to the subfamily POLYCOTYLINAE.

Any real attempt to evaluate the more than usual elaborate system of classification as proposed by Dubois (1936a, 1936b, 1938b) for the strigeid trematodes does not seem necessary at this time. We are more particularly concerned with those forms reported from the REPTILIA, and since all of our material came from a single host species, *Alligator mississippiensis*, we do not feel justified in expressing an opinion concerning the



multitude of genera proposed for the relatively small number of species from these hosts. It does seem logical, however, to place this rather homogeneous assemblage of parasites in a single family as Dubois already has done. With but two possible exceptions among the more adequately described species all members of the family group possess a common character in the presence of the prostate gland, a structure not to be found elsewhere among the multitude of genera and species of strigeid trematodes.

In general the topography of the genital ducts in the vicinity of the genital atrium is rather simple. The ventrally located uterus descends to the atrium as a thin walled, slightly modified or unmodified tube, and usually opens into the atrium at the base of a more or less conical papilla. The ejaculatory duct descends, just dorsal to the uterus, from the seminal vesicle to near the atrium where it usually discharges into the uterus, thus forming a hermaphroditic duct. The prostate normally descends into the genital papilla and opens into the atrium at the summit of the papilla. This simple arrangement of the genital ducts is sometimes greatly modified, and is always difficult to trace in whole mounts.

In the genus *Polycotyle* (Fig. 1, E) the uterus develops a small, though well outlined sphincter muscle before it is joined by the ejaculatory duct. The duct created by the fusion of these two ducts traverses only a short part of the genital papilla before fusing with the prostatic canal; the new duct penetrates the genital papilla to open into the atrium at its summit. In this genus, therefore, a slight change in the association of the genital ducts has been effected: the uterus no longer opens at the base of the papilla but joins the prostatic canal before discharging into the atrium.

As already pointed out, the association of the genital ducts in the material identified as *Crocodilicola pseudostoma* by us differs from that described by other workers for the same species. We find that the ejaculatory duct (Fig. 1, F) no longer discharges into the uterus but has become associated with the prostatic canal as is the case with the members of the genus *Pseudoneodiplostomum*. The uterus does unite with the

FIG. 1. Schematic representation of a probable evolutionary line of development of the prostate gland in the family PROTERODIPLOSTOMIDAE of the strigeid flukes. The type of development of the prostate together with its association with the other ducts of the genital tract may indicate the function of the organ. A = *Mesodiplostomum*; B = *Paradiplostomum*, *Herpetodiplostomum*, etc.; C & D = *Prolecithodiplostomum*, *Cystodiplostomum*, *Ophiodiplostomum* (?), etc.; E = *Polycotyle ornata*; F & F' = *Crocodilicola*; G = *Pseudoneodiplostomum acetabulata*; H = *Pseudocrocodilicola americanense*; I = *Pseudocrocodilicola georgiana*; J = *Pseudoneodiplostomum thomasi*; K = *Pseudoneodiplostomum siamense*; L = *Proterodiplostomum*; M = *Heterodiplostomum*; and O = *Pharyngostomum cordatum*. *Pharyngostomum cordatum* is included in the schematic diagram solely because of the evolutionary significance of its atrial recess.

prostatic canal and both discharge into the atrium through a common opening. It might be noted that the ejaculatory duct in the species *Pseudoneodiplostomum siamense* (Poirier, 1886) (Fig. 1, K) is surrounded by a mass of prostatic gland cells, the prostate being absent or only weakly developed. It will be remembered, the new species, *P. acetabulata* (Fig. 1, G), differs from the other two members of the genus *Pseudoneodiplostomum*, *P. thomasi* (Dollfus, 1935) (Fig. 1, J) and *P. siamense* (Poirier, 1886), in that the uterus joins the prostatic canal instead of opening separately from the prostatic canal as is the case with the other two species.

In the species *Pseudocrocodilicola americanense* (Fig. 1, H) the ejaculatory duct joins the prostatic canal at about the middle of the prostate. The canal so formed discharges into the dilated part of the cavity of the secondary pouch, just dorsal to the opening of the uterus. In this species the proximal end of the uterus shows a modification of its wall in that the spongy-like tissue, mixed with muscle fibers, of the secondary pouch forms a short continuation of its tissue, and this surrounds the wall of the uterus. However, there is evidently no such continuation of the secondary pouch in the make up of the uterine wall in the area of the pouch in *P. georgiana* (Fig. 1, I). In this species, however, the ejaculatory duct joins with the prostatic canal at its extreme anterior end and both the uterus and prostate penetrate the wall of the secondary pouch to discharge simultaneously into the dilated portion of the cavity of the secondary pouch. A probable significance of the secondary pouch will be discussed later.

It is our opinion that the prostate gland is a definite, recognized feature of the anatomy within the family PROTERODIPLOSTOMIDAE. Its nature and presence was suggested by Willemoes-Suhm (1870), Poirier (1886) and others but its true anatomy and possible significance perhaps was first indicated by Brandes (1890). Of the later workers, Dubois (1936b, 1938b) has done much to elucidate the details concerning the anatomy and relative connections of the prostate with the other genital ducts. As yet, however, the exact nature and function of the gland is more a matter of conjecture than an established fact.

Perhaps La Rue (1926a) came nearer to recognizing the way in which the prostate originated than had been previously indicated. In discussing the anatomy of *Pharyngostomum cordatum* (Diesing, 1850) (Fig. 1, O), this writer made this statement: "A slender recess (Fig. 5) extends from the genital atrium nearly to the seminal vesicle. A similarly situated structure is figured by Brandes (1890) in *Diplostomum abbreviatum* and in certain other forms, and by him is called the prostate gland. In the present species there is no indication of gland cells about this structure but there are well developed muscle fibers. I suspect that the prostate

described and figured by Brandes is not a prostate at all but a cavity of an eversible portion of the genital papilla." Whether or not an eversible portion of the genital cone has been demonstrated is more a matter of personal interpretation, although the existence of such a structure is highly probable. To us the prostate, as portrayed in this group of the strigeid flukes, undoubtedly represents a structure "on the way in" or one that is "on the way out." From an evolutionary point of view it does not matter which of the two alternatives one selects for argumentation. The slender recess of the genital atrium in *Pharyngostomum cordatum* undoubtedly represents simply a part of the genital atrium as is evidenced by the fact that its cavity is in open communication with the bursal portion of the atrium, and by the fact that the cavity is surrounded by a more or less dense mass of muscle fibers: such muscle fiber masses are present in tissue forming the bursa of all strigeid flukes known to us. However, the position of the recess, its length and its relation to the genital ducts would indicate its evolutionary significance. Should one be disposed to take the stand that the prostate is "on the way in," such a recess easily might represent the primordium of the gland. On the other hand, it might represent the vestigial remains of a prostate "on the way out." Certainly some such invagination as this recess formed at least the terminal part of the prostate. The cuticular lining of the central cavity of the gland would indicate such an origin.

Perhaps the invaginated recess of the prostate reaches its highest degree of specialization in the genus *Polycotyle*. Here the gland-like organ of the prostate (Fig. 1, E) has developed into an elongated, tubular structure with a heavy muscular wall and is surrounded by numerous, unicellular gland cells. These are rather large cells (Pl. II, Fig. 4) that show conspicuous nuclei and evacuated cytoplasm. Each of the gland cells gives rise to a very fine duct that penetrates the musculature of the prostate to open into its lumen. The cells are more concentrated about the upper two-thirds of the prostate. The cavity of the gland is lined with a cuticular-like substance that becomes ridged to give it a corrugated appearance. It might be pointed out that the invagination of the prostate may become more extensively developed than is the case with the genus *Polycotyle*. In the genera *Prolecithodiplostomum* and *Cystodiplostomum* (Fig. 1, C & D) the prostate develops into a much elongated tubule and may extend forward as far as the caudal testis. However, in these genera the prostate appears to be a simple tube that does not reach the degree of specialization noted for the organ in *Polycotyle*. Such a degree of specialization is not attained in any of the other genera of the family with the possible exceptions to be found in the genera *Pseudocrocodilicola* and *Crocodilicola*. In the latter genus the cells about the prostate are much smaller and much less numerous, while in the former genus these

gland cells appear to be more like vitelline glands in comparison to the type of cell to be found in *Polycotyle*. The prostatic gland cells in the genus *Pseudocrocodilicola* appear to be filled with dark-staining granules and are more thinly dispersed in the parenchyma about the prostate and other genital ducts in the area.

As already suggested the prostate probably represents an invaginated part of the atrium. However, if the prostate, as it is recognized at the present time, is in any way comparable with any part of the cirrus pouch of the distomes, it is only feebly indicated in this group of the strigeids. The muscular nature of the organ, as well as its communicating gland cells, when these are present, would suggest that it is an accessory piece in the genital complex, and that it undoubtedly serves some ejaculatory or storage function. The ejaculatory function is made manifest when we consider those species in which the ejaculatory duct penetrates the musculature of the prostate to discharge into its cavity. Such an association is best exemplified in the species *Pseudoneodiplostomum thomasi* (Dollfus), *P. siamense* (Poirier), *P. acetabulata* n. sp., *Pseudocrocodilicola americanense* n. sp. and *P. georgiana* n. sp. Should the condition described for the prostate and ejaculatory duct in *Pseudoneodiplostomum siamense* (Fig. 1, K) be established as correct, no other interpretation can be made but that the weakly muscular prostate with its numerous gland cells serves the ejaculatory duct in performing the function of discharging the male gametes. In this species the prostate is reduced to practically a mass of gland cells that surrounds the ejaculatory duct. In the species *Pseudocrocodilicola georgiana*, although the prostate is highly muscular but supplied with relatively few gland cells, the ejaculatory duct (Fig. 1, I) enters the prostatic canal at its anterior extremity. The spermatozoa, therefore, are discharged into the prostatic canal and must reach the outside only after traversing the entire length of the prostatic canal. In this species the prostatic canal and prostate frequently are seen to be greatly dilated and filled with male gametes, thus indicating that the prostate at least serves as a temporary storage space for the semen prior to ejaculation. Likewise, it is our observation that the prostatic canal in the other species already mentioned often become distended with spermatozoa. In these cases the musculature of the prostate must in some way serve as a pars prostatica in the expulsion of the male germ cells. We might add that the prostatic canal of *Polycotyle ornata* was not observed to contain spermatozoa but was filled with a fluid that had been coagulated by the fixing agent. In this species it will be remembered that the ejaculatory duct joined the uterus rather than the prostatic canal.

Perhaps the more primitive type of arrangement of the terminal portions of the genital ducts and their association with the prostate gland is best exemplified in the species *Paradiplostomum abbreviatum*. Here we

find a very small prostate (Fig. 1, B) that is confined to the genital papilla and bearing but very few gland cells. The prostate opens into the atrium at the summit of the papilla, while the ejaculatory duct fuses with the uterus and these two ducts discharge together into the atrium some distance from the opening of the prostate. It is quite likely that the more primitive condition would exclude the prostate entirely, as in *Mesodiplostomum gladiolum* Dubois (Fig. 1, A).

From the type of association as found in *Paradiplostomum abbreviatum* we are of the opinion that a dual migration of the uterus and ejaculatory duct has taken place. Along one line the uterus and ejaculatory duct have migrated together so that the short hermaphroditic canal formed by the fusion of these two ducts no longer opens directly into the atrium but fuses with the prostatic canal. Such a migration as this can be illustrated in *Polycotyle ornata* (Fig. 1, E) wherein the ejaculatory duct and uterus fuse into the formation of the hermaphroditic canal, and this in turn fuses with the prostatic canal. In this species only a single opening serves all of the genital ducts as well as the prostate. In pursuing this line further, the association found in the species *Pseudoneodiplostomum acetabulata* (Fig. 1, G) and *P. thomasi* (Fig. 1, J) presents evidence of a shift in the connection of the ejaculatory duct with the uterus to one in which that duct migrates across and becomes fused with the prostatic canal. This type of migration is perhaps perfected in *Pseudoneodiplostomum siamense* (Fig. 1, K) in which the ejaculatory duct penetrates the entire length of the reduced prostate. It is to be pointed out that the uterus in *Pseudoneodiplostomum thomasi* and *P. siamense* opens into the atrium independently of the prostatic canal. This may mean a lagging behind in the migration of the uterus or a backward shift.

The condition found in the genus *Pseudocrocodilicola* probably represents the full development in the shifting of the uterus and ejaculatory duct from an independent position to one of association with the prostate. In this genus the hermaphroditic canal has developed its musculature into what we have called a secondary pouch, and has become somewhat dilated into the formation of a genital cloaca. Very likely this development has taken place at the expense of the genital atrium for this formation is greatly reduced in the genus. Into the dilated cavity of the secondary pouch both the uterus and prostatic canal discharge. The ejaculatory duct in *P. americanense* (Fig. 1, H) fuses with the prostatic canal at about the middle of the prostate, thus putting the prostate in line for a pars prostatic function. In the species *P. georgiana* (Fig. 1, I) such a function for the prostate is made more possible since the ejaculatory duct discharges into the prostatic canal at its anterior extremity.

Concerning the secondary pouch in the genus *Pseudocrocodilicola*, there appears to be no structure so far described among the strigeids that

is in any way comparable to it. In describing the species *Heterodiplostomum lanceolatum*, Dubois (1936b) described a cirrus pouch. The prostate is small but penetrates the cirrus pouch at its anterior end, and the prostatic canal ramifies the pouch before opening to the outside at the summit of the genital papilla. The association of the ejaculatory duct is with the uterus rather than with the prostatic canal, and these two ducts open into the atrium independently of the prostate. The association of the genital ducts and the presence of an eversible cirrus, an organ not observed in any of our material, in this species (Fig. 1, M) completely separates it from the material studied by us.

With the possible exception of the members of the subfamily POLYCOTYLINAE and the genus *Proterodiplostomum* all of the species described or studied by Dubois (1936b) exhibited an ejaculatory duct that joined the uterus to form a short hermaphroditic canal. This canal discharged into the genital atrium independently of the prostate. Likewise all of the species described by Dubois possessed a prostate with but one exception. The species *Mesodiplostomum gladiolum* (Fig. 1, A) is completely devoid of any structure that may be comparable with the prostate. The exact association of the genital ducts or the nature of the prostate, if such a structure exists, is not known for the species *Proalarioides serpentis* Yamaguti, 1933.

There is but little evidence pointing to the second line of evolution in this group of the strigeids. This line takes the direction of separating the uterus from the male genital duct and the prostate. In this group, represented by the *Proterodiplostomum* (Fig. 1, L), the uterus, ejaculatory duct and prostate may open separately into the atrium or the uterus opens into the atrium at the base of the genital papilla while the ejaculatory duct and prostatic canal open together at the summit of the papilla. As a matter of fact, this condition may represent an intermediate position between the pattern in *Paradiplostomum abbreviatum* and that displayed by *Pseudocrocodilicola georgiana*.

SUMMARY

The detailed anatomy is described for *Polycotyle ornata* Willemoes-Suhm, 1870, *Crocodilicola pseudostoma* (Willemoes-Suhm, 1870), *Pseudoneodiplostomum acetabulata* n. sp., *Pseudocrocodilicola americanense* n. sp. and *P. georgiana* n. sp. The last three named species are considered and described as new to science. All of the material considered by us came from the small intestine of the alligator, *Alligator mississippiensis* (Daudin).

The genus *Pseudocrocodilicola* is proposed as new. It is placed in the subfamily POLYCOTYLINAE Monticelli, 1888.

The evolution of the terminal portions of the genital ducts and the development of the prostate gland in the family PROTERODIPLOSTOMIDAE,

strigeid parasites of reptiles, is discussed with the view of throwing some light on the origin and function of the prostate gland.

BIBLIOGRAPHY

- BRANDES, G. 1890 Die Familie der Holostomiden. Zool. Jahrb. (Syst.) 5: 549-604.
- DOLLFUS, R. PH. 1935 Sur *Crocodilicola* et autres Hémistomes de Crocodiliens. Arch. Mus. Hist. Nat. 12: 637-646.
- DUBOIS, G. 1936a Nouveaux principes de classification des Trématodes du groupe des Strigeida. Rev. Suisse Zool. 43: 507-515.
- 1936b Les Diplostomes de Reptiles (Trematoda: Proterodiplostomidae nov. fam.) du Musée de Vienne. Bull. Soc. Neuchatel Sc. Nat. 61: 5-80.
- 1938a Liste systématique des Strigéidés du Brésil et du Venezuela. Livro Jubilar Prof. Travassos. III. pp. 145-155.
- 1938b Monographie des Strigeida (Trematoda). Mem. Soc. Neuchatel Sc. Nat. 6: 5-535.
- LA RUE, G. R. 1926a Studies on the trematode family Strigeidae (Holostomidae) No. I. *Pharyngostomum cordatum* (Diesing) Ciurea. Tr. Am. Micr. Soc. 45: 1-10.
- 1926b Studies on the trematode family Strigeidae (Holostomidae) No. II. Taxonomy. Tr. Am. Micr. Soc. 45: 11-19.
- 1926c Studies of the trematode family Strigeidae (Holostomidae) No. III. Relationship. Tr. Am. Micr. Soc. 45: 265-281.
- MONTICELLI, F. S. 1888 Saggio di una morfologia dei Trematodi (Tesi). Napoli, 130 pp.
- NARAIN, D. 1930 *Neodiplostomum gavialis* n. sp. from the crocodile. J. Parasitol. 16: 154-157.
- POIRIER, J. 1886 Sur les Diplostomidae. Arch. Zool. Expér. et Gén. 4: 327-346.
- WILLEMOES-SUHM, R. VON 1870 Ueber einige Trematoden und Nemathelminthen. Diss. (Göttingen), 29 pp.
- 1871 Ueber einige Trematoden und Nemathelminthen. Z. Wissensch. Zool. 21: 175-203.
- YAMAGUTI, S. 1933 Studies on the Helminth Fauna of Japan. Part 1. Trematodes of birds, reptiles and mammals. Japan. J. Zool. 5: 1-134.

EXPLANATION OF PLATES

Except for certain details of the terminal parts of the genital ducts all illustrations of the entire worm were made with the aid of the camera lucida. All illustrations of the posterior segment of the body are reconstructions made from serial sections.

ABBREVIATIONS USED

A—atrium	PC—prostatic gland cells
AS—atrial sucker	SG—shell gland
C—cecum	SM—sphincter muscle of uterus
DS—dorsal sucker	SP—secondary pouch
EJ—ejaculatory duct	SR—seminal reservoir
EP—excretory pore	SV—seminal vesicle
GP—genital papilla	T ₁ —anterior testis
HD—hermaphroditic canal	T ₂ —posterior testis
LC—Laurer's canal	UT—uterus
M—metraterm	VD—vas deferens
MEJ—muscular portion of ejaculatory duct	VE ₁ —vas efferentia of anterior testis
O—ovary	VE ₂ —vas efferentia of posterior testis
OV—ova	VIT—vitellaria
P—prostate	YD—yolk duct
	YR—yolk reservoir

PLATE I

FIG. 1. *Pseudoneodiplostomum acetabulata* n. sp., showing general contour of the body, the position of the various internal organs and the associations of the genital ducts with the prostate. Ventral view.

FIG. 2. *Pseudoneodiplostomum acetabulata* n. sp., showing reconstructed arrangement of the genital organs in the posterior segment of the body. The extreme posterior end of the body is made more detailed in order to show the associations of the genital ducts with the prostate, the length of the ceca, the metraterm and the posterior distribution of the vitellaria. Diagrammatic. Greatly enlarged. Lateral view.

PLATE II

FIG. 3. *Polycotyle ornata* Willemoes-Suhm, 1870, showing general contour of the body, the position of the internal structures and the dorsal row of suckers. Anterior segment in ventral view. Posterior segment in lateral view.

FIG. 4. *Polycotyle ornata* Willemoes-Suhm, 1870, showing reconstructed arrangement of the genital organs and the associations of the genital ducts with the prostate. The posterior end of the body is shown in some detail so that the prostate, the prostatic gland cells, the sphincter muscle of the uterus, the union of the ejaculatory duct with the uterus, the atrial sucker, the termination of the cecum and the last two suckers of the dorsal row with the two very small suckers in between them could be made more clear. Diagrammatic. Greatly enlarged. Lateral view.

PLATE III

FIG. 5. *Crocodilicola pseudostoma* (Willemoes-Suhm, 1870), showing details of the body and the arrangement of the genital organs. Ventral view.

FIG. 6. *Crocodilicola pseudostoma* (Willemoes-Suhm, 1870) showing reconstructed view of the organs in the posterior half of the body. Note the size and shape of the prostate, the arrangement of the prostatic gland cells, the muscular wall of the ejaculatory duct and its connection with the prostate, and the muscular sphincter at the end of the uterus. Diagrammatic. Greatly enlarged. Ventral view.

PLATE IV

FIG. 7. *Pseudocrocodilicola americanense* n. g., n. sp., view of entire worm showing general contour of the body and the arrangement of the various internal organs. Ventral view.

FIG. 8. *Pseudocrocodilicola americanense* n. g., n. sp., reconstruction of genital organs in posterior segment of the body. The extreme posterior margin of the body is represented more in detail in order to show the small atrium, the secondary pouch, the continuation of the pouch along the proximal part of the uterus, the prostate and its connection with the ejaculatory duct, the prostatic gland cells and the termination of the ceca. Diagrammatic. Greatly enlarged. Ventral view.

PLATE V

FIG. 9. *Pseudocrocodilicola georgiana* n. g., n. sp., view of entire worm showing general contour of the body and the arrangement of the internal anatomy. Dorsal view.

FIG. 10. *Pseudocrocodilicola georgiana* n. g., n. sp., reconstruction of organization in the posterior segment of the body. Compare the anatomy of this species with that of *P. americanense*. Diagrammatic. Greatly enlarged. Ventral view.

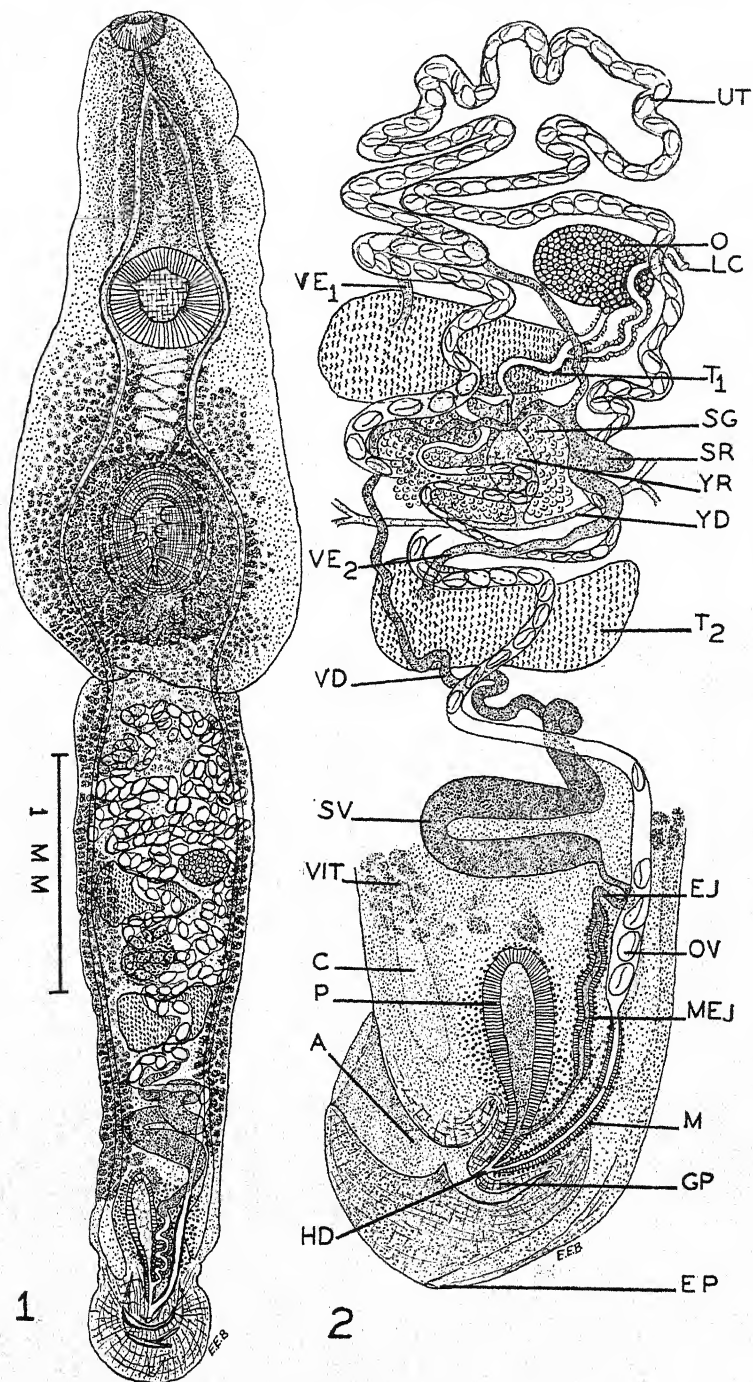


PLATE I

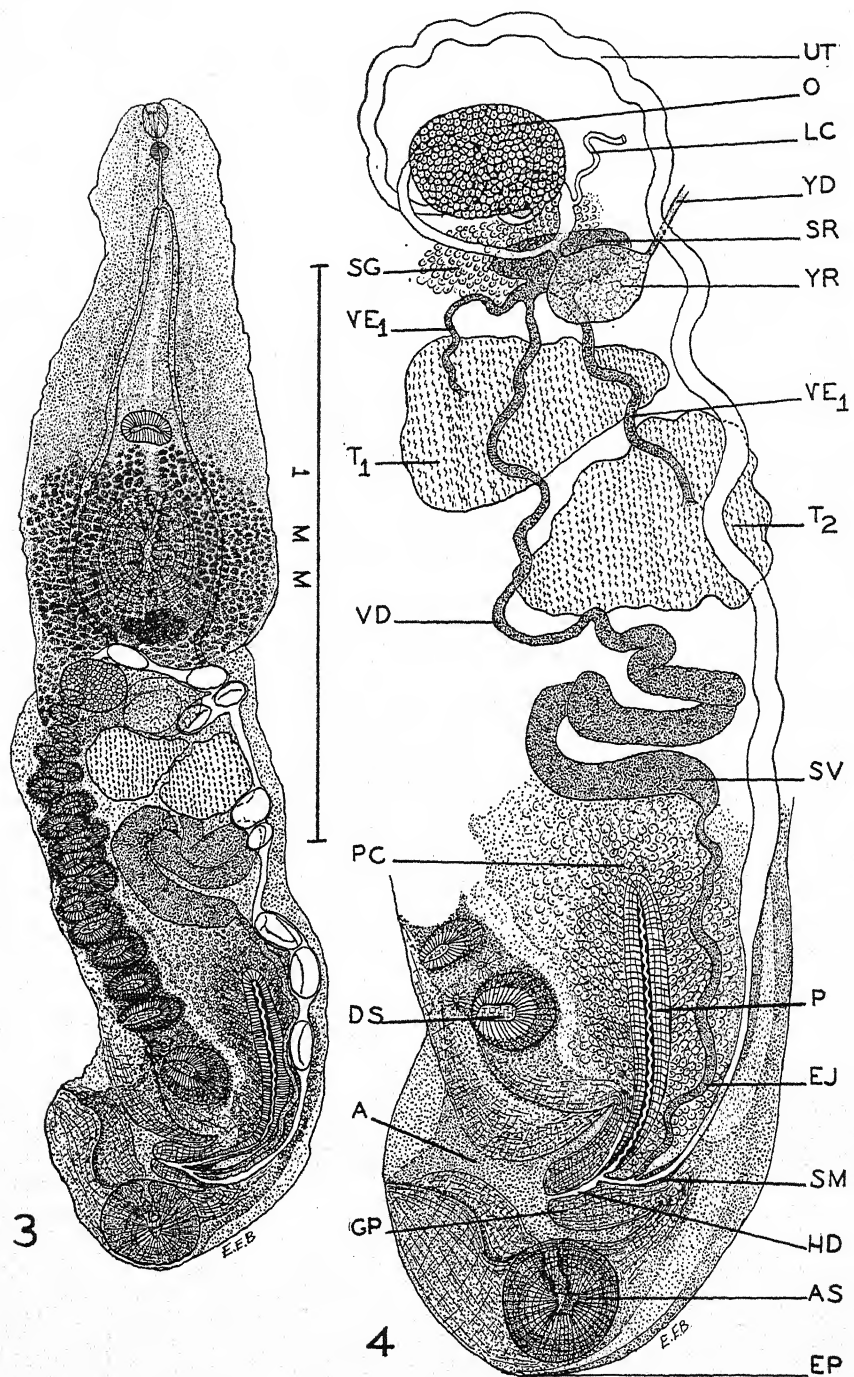


PLATE II

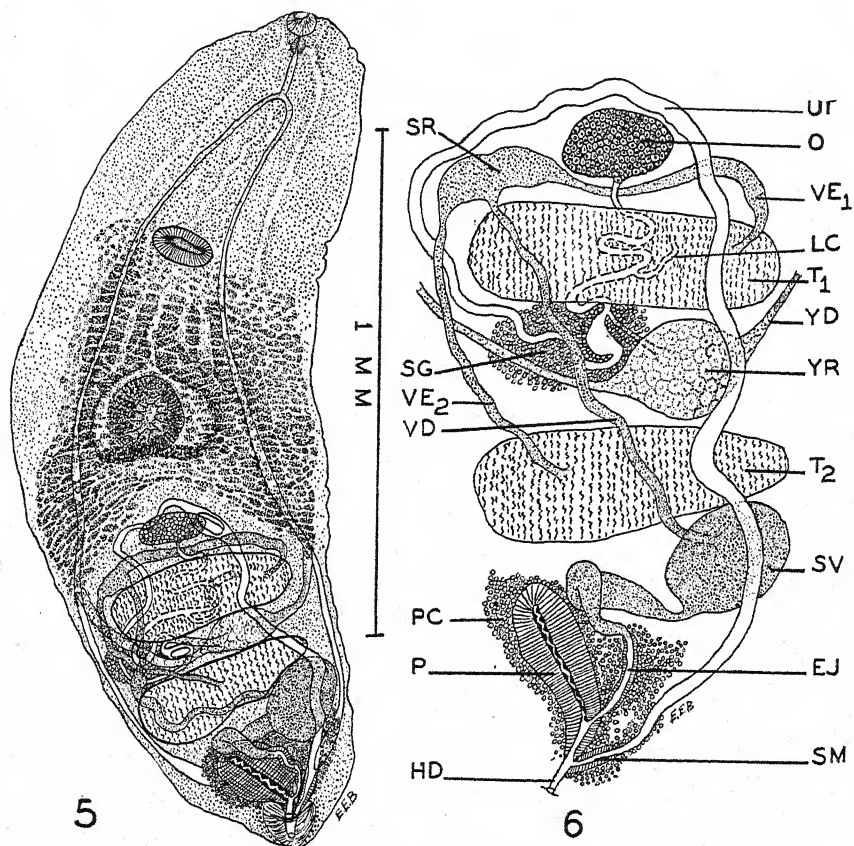


PLATE III

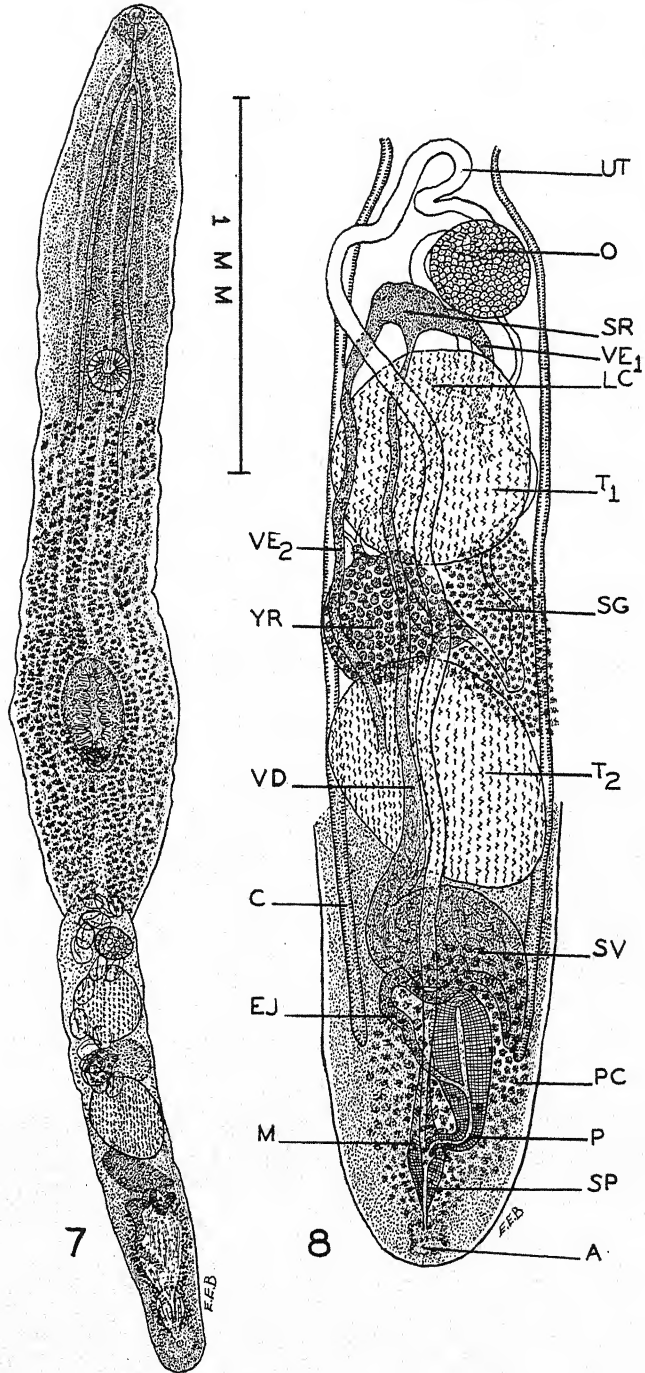


PLATE IV

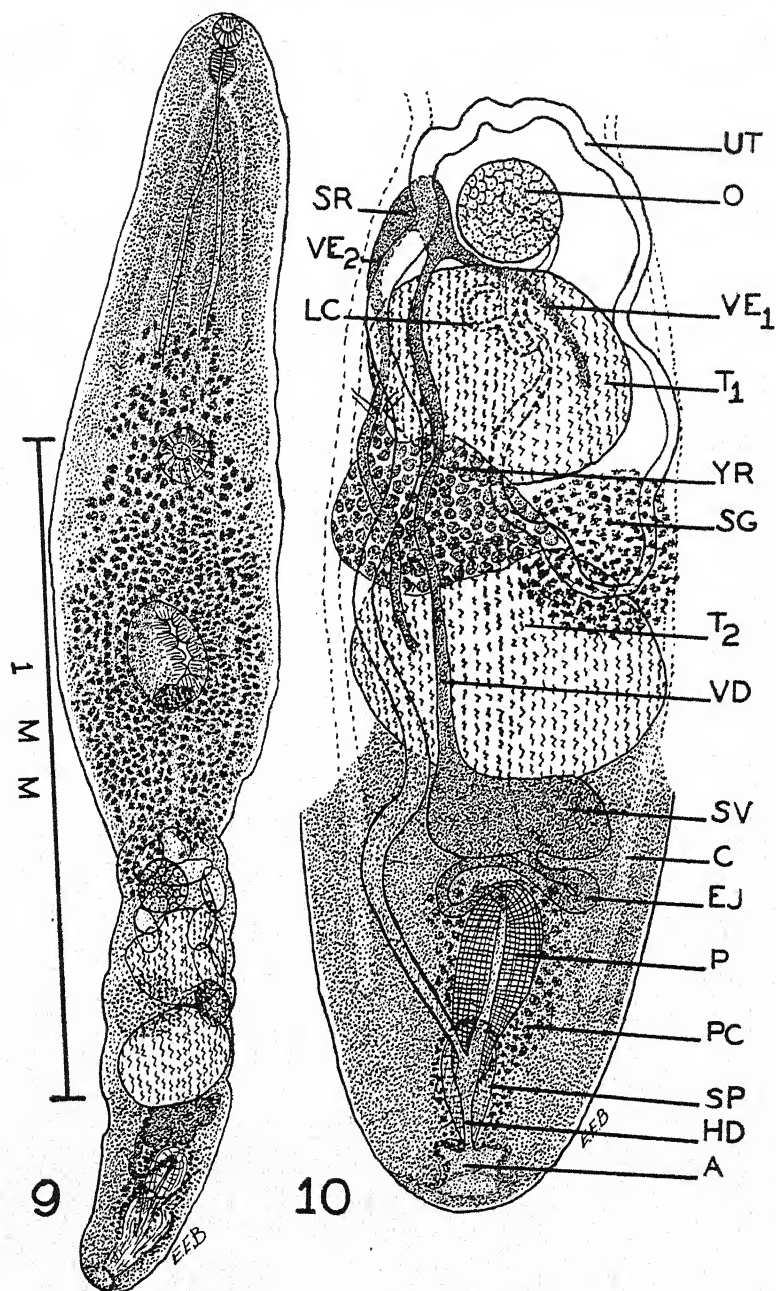


PLATE V

AN EXPERIMENTAL TEST OF THE LIFE CYCLE
DESCRIBED FOR *COTYLURUS COMMUNIS*
(HUGHES)*

LOUIS OLIVIER AND W. W. CORT

On the basis of experiments conducted in 1926 and 1927, Van Haitsma (1930) presented a description of the life cycle of a strigeid trematode designated by him as *Cotylurus michiganensis*. Later, La Rue (1932) pointed out that the correct name for the species is *Cotylurus communis* (Hughes, 1928). Van Haitsma demonstrated by feeding experiments that strigeid metacercariae identified as *Tetracotyle communis* Hughes found on and about the hearts of common suckers, *Catostomus commersonnii* (Lacépède), became adults of *C. communis* in the bursa Fabricii of the herring gull, *Larus argentatus* (Pont.). He then collected eggs from the bursae of the experimentally infected gulls and allowed them to embryonate. Seven species of snails were exposed to the miracidia which developed. One of the snails, a specimen of *Lymnaea emarginata angulata* Sowerby (= *Stagnicola emarginata angulata*), produced large numbers of strigeid cercariae forty days later. This snail had been collected from Douglas Lake and kept in the laboratory three weeks before it was exposed to the miracidia. The intestine of the gull, from which the eggs used in this particular experiment were obtained, harbored two specimens of a species of *Diplostomum*. The bursa contained many specimens of *C. communis*. Van Haitsma concluded that the single snail mentioned had been infected experimentally and that the cercariae belonged to *C. communis*. He stated that similar experiments conducted in 1928 yielded the same results but he did not describe these experiments.

Cort and Brackett (1937) considered the cercariae obtained by Van Haitsma to be identical with *C. emarginatae* Cort, 1917 which was described from the same host in the Douglas Lake Region.

Szidat (1931, p. 166) questioned the validity of the life cycle of *C. communis* as outlined above. He believed that the location of the penetration glands in the cercariae of members of the same genus should be similar. Since the penetration glands of Van Haitsma's cercaria lie behind the ventral sucker while those of the cercaria of *C. cornutus* are in the front of the ventral sucker, he felt that the two species were prob-

Received for publication, March 7, 1941.

* Contribution from the University of Michigan Biological Station; the Biological Laboratory, New York University, University Heights; and the Department of Helminthology, School of Hygiene and Public Health, the Johns Hopkins University.

ably not closely related. Consequently, he suggested that Van Haitsma's cercaria might belong to the species of *Diplostomum* that was in the intestine of the bird from which the eggs used in the experiment were taken. Dubois (1938) also questioned the validity of this part of the life cycle. He noted that the snail in which the cercariae appeared had been in the laboratory only three weeks before exposure to the miracidia. This seemed to him an insufficient time to preclude the possibility of previous infection.

Since these workers had seriously questioned the correctness of the life cycle described for *C. communis*, it seemed desirable to throw further light on the matter. Therefore, we attempted to complete experimentally that part of the cycle not carried through by Van Haitsma, namely, the infection of the second intermediate host with the subsequent recovery of the mature metacercariae. If *Tetracotyle communis* could be recovered from fish after they had been exposed to Van Haitsma's cercaria, the doubt concerning the validity of the described life cycle would be removed.

INFECTION EXPERIMENTS

The cercaria described by Van Haitsma (1930) as belonging to *C. communis*, and originally described as *C. emarginatae*, is common in the Douglas Lake region. Its structure is very characteristic and it can be readily distinguished from all other strigeid cercariae which occur in the region. Several specimens of *S. emarginata angulata* harboring infections with this cercaria were used as the source of the cercariae for the infection experiments. In order to make certain that these cercariae were identical with those of Van Haitsma, their morphology was worked out in detail and checked against his description. No differences were discovered, but certain additional morphological information was secured which will be given later. We are certain, therefore, that the cercariae which we used in the following infection experiments belonged to the same species as those which Van Haitsma described as belonging to *C. communis*.

In a preliminary experiment two perch (*Perca flavescens* (Mitch.)) from Vincent Lake were exposed to these cercariae. Vincent Lake is an acid lake which harbors no snails and no trematodes have ever been found in fish taken from it. The two perch were placed in an aquarium supplied with a small stream of lake water and water containing cercariae was added on July 25, 1940. On July 27, one of the fish was dissected and metacercariae with all the characteristics of the cercarial body were recovered from both eyes. Additional cercariae were added to the aquarium on August 1, 12, and 20. The second fish was examined on August 22 and numerous metacercariae of the diplostomulum type in four distinct stages of development were recovered from both eyes. It was evi-

dent, therefore, that the metacercariae recovered had been introduced experimentally and were 2, 10, 21, and 28 days old. Three perch of the same collection were maintained under identical conditions in another aquarium but were not exposed to cercariae. No metacercariae were found in these fish on August 22.

Subsequently, we conducted three additional experiments involving a total of fourteen perch from Vincent Lake. Cercariae were introduced into the tanks periodically as in the above experiment. Metacercariae were recovered from the eyes of all the fish after from two to 26 days. In one of the experiments only well water was used to preclude the possibility of contamination from the lake water.

One bull-head (*Ameiurus nebulosus* (LeSueur)), five blue-gills (*Lepomis macrochirus* (Rafinesque)), four rock bass (*Ambloplites rupestris* (Rafinesque)), and three log perch (*Percina caprodes* (Rafinesque)) were also exposed to the cercariae but none became infected.

The results of these experiments demonstrate that the cercaria described by Van Haitsma as belonging to *C. communis* penetrates into perch and develops in the eyes into a typical diplostomulum. Therefore, it can have no relationship to the life cycle of *C. communis* since the metacercaria of this species is a tetracotyle found around the hearts of fishes. This finding indicates that Szidat and Dubois were correct in questioning the validity of Van Haitsma's experiments. Either the snails he used were previously infected or were infected with miracidia from the eggs of diplostomes present in the intestine of his gull host. Therefore, the species of cercaria which Van Haitsma (1930) mistakenly connected with *C. communis* should now be known as *Cercaria emarginatae* Cort, 1917.

DATA ON *C. emarginatae* AND ITS METACERCARIAL STAGE

The original description by Cort (1917) of *C. emarginatae* is very incomplete but Van Haitsma's later description is given in considerable detail. As noted above, a careful study of the cercariae used in our experiments failed to reveal differences between his description and our material. However, we are able to add a few details to the description as a result of our studies. We will also include descriptions of the immature and mature metacercariae which we obtained from the experimentally infected fish, followed by information we have gathered concerning the host-parasite relations of the metacercariae.

Additions to the description of C. emarginatae. The arrangement of the excretory tubules, not determined by Van Haitsma (1930, fig. A), appears in Fig. 1. The proximal portion of the posterior collecting tubule bears three patches of cilia. The ventral body spination is shown

in Fig. 1. About nine transverse rows of spines occur in the region between the circum-oral band of spines and the level of the ventral sucker. Scattered spines occur among the first three or four transverse rows. The ventral surface behind the acetabulum is covered with scattered spines except for a small median spineless area. The dorsal body spination is identical except that no spines occur posterior to the level of the ventral sucker and the spines at the level of the pharynx are more sparse. Six to nine hooked "forward projecting spines" always appear

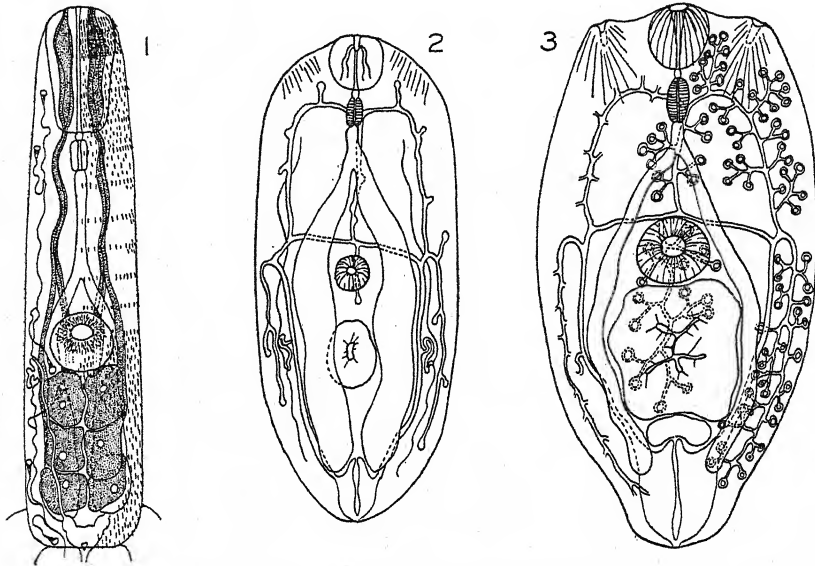


FIG. 1. Body of *Cercaria emarginata* to supplement Fig. A in Van Haitsma (1930). Ventral aspect. Excretory system is shown only on the left; spination only on the right.

FIG. 2. Developing metacercaria (10-day-larva) of *C. emarginata* from eye of perch. Ventral aspect. Contents of ceca not shown. Note the developing reserve excretory system, small holdfast, and remnants of gland ducts in the partially metamorphosed oral sucker.

FIG. 3. Mature metacercaria (28-day-larva) of *C. emarginata* from eye of perch. Ventral aspect. To the same scale as Fig. 2. Reserve excretory system is incompletely shown on left.

immediately dorsal to the oral opening. The spines on the ventral sucker and in the circum-oral band are also hooked. The intestinal ceca are very prominent and the esophagus and portions of the ceca lying anterior to the acetabulum are usually expanded so that under low magnification there appears to be a large clear area in front of the ventral sucker. Twenty-five specimens fixed by the usual method (Cort and Brackett, 1937) were measured and the results are as follows:

	Range (mm)	Mean with P.E. (mm)
Body length	0.221-0.268	0.253 \pm 0.002
Body width	0.040-0.047	0.046 \pm 0.001
Tail stem length	0.308-0.368	0.344 \pm 0.002
Tail stem width	0.034-0.047	0.040 \pm 0.001
Furcal length	0.288-0.335	0.315 \pm 0.002
Oral sucker length	0.056-0.069	0.062 \pm 0.001
Oral sucker width	0.021-0.027	0.024 \pm 0.001
Ventral sucker diameter	0.024-0.029	0.027 \pm 0.001

Description of immature metacercariae. Larvae recovered two days after penetration retained all the characteristics of the cercarial body and some of the details of the cercarial structure were checked by studying them. It is noteworthy that in these young larvae the excretory system and spination were unchanged and only a small part, if any, of the contents of the penetration glands had been lost. The larvae reached the eyes in large numbers within two days after penetration.

Larvae ten days old were obviously immature but had undergone considerable morphological change (Fig. 2). The oral sucker was partially changed though the gland ducts in it could still be seen. The main channels of the reserve excretory system had developed and some of the smaller branches had appeared. The holdfast was small but had a lumen. Lateral sucking areas were beginning to develop on either side of the oral sucker. The digestive ceca were very conspicuous since they were large and contained much black pigment in the form of small rods and granules. The hind body was just beginning to form. Thirteen worms fixed at this stage were 0.36-0.44 mm long and 0.11-0.20 mm broad. Thirteen and fourteen day old metacercariae were still very similar to the ten-day larvae.

Larvae twenty-one days old had almost completed their metamorphosis. The only indication of morphological immaturity was that the excretory concretions were noticeably smaller than those of older metacercariae.

Description of mature metacercariae. The oldest larvae secured in the experiments were twenty-eight days old. They had all the characteristics of typical diplostomula and were apparently mature morphologically (Fig. 3). The forebody was flat and broad. The holdfast organ was large, filling the space between the ventral sucker and the excretory bladder. The lateral glandular areas which lie on either side of the oral sucker were conspicuous and were usually protruded slightly in living specimens. In fixed specimens they often appeared as cup-like lateral depressions. The form and extent of the reserve excretory system are shown in Fig. 3. The concretions at the ends of the channels varied from 3 to 12 microns in diameter and were usually spherical although some were oblong or irregular. The intestinal ceca contained no pigment and were relatively narrow and inconspicuous. Numerous,

small gland-like structures were observed beneath the cuticula on the anterior two-thirds of the worms. Fifteen fixed specimens were 0.37–0.49 mm long and 0.14–0.25 mm broad. The diameter of the oral sucker in ten specimens ranged from 0.036 to 0.041 mm and the diameter of the ventral sucker from 0.038 to 0.050 mm. The pharynx in seven fixed specimens was 0.036 to 0.043 mm long and 0.019 to 0.024 mm broad. Although it is not expected that older metacercariae of this species will be found to differ appreciably in structure, they may be larger than the metacercariae just described, since as pointed out by Cort and Brackett (1938), strigeid diplostomula may increase in size for some time after reaching apparent maturity. It may also be added that some strigeid larvae are not infective when they reach what may be called morphological maturity but require an additional period during which no evident morphological changes occur (Olivier, 1940, p. 456).

Location of metacercariae in the second intermediate host. As stated previously, dissection of experimental fish always revealed that the metacercariae develop exclusively in the eyes. Eyes containing metacercariae of different ages were fixed and sectioned. Study of this material revealed that almost all of the metacercariae were located in the retina between the sensory cells and the pigmented layer. Only two were found elsewhere; one was in the optic nerve near its exit from the eye ball, and the other in the layer of ganglion cells of the retina.

Damage to the retina was usually limited to the sensory cells immediately adjacent to the parasites. One of the eyes which harbored two-day-larvae had a large quantity of blood in a space apparently created by the parasites between the pigmented layer of the retina and the layer of sensory cells. In the other eyes there was no indication of hemorrhage or of a defense reaction to the parasites.

Evidently the young metacercariae feed on the tissue of the retina since the ceca of the ten, thirteen, and fourteen day larvae were filled with black material which was obviously retinal pigment. The ceca of older larvae, twenty-one to twenty-eight days after penetration, contained no pigment. It seems likely that these older larvae had stopped actively ingesting food and probably were securing their nourishment by osmosis through the body wall since their ceca were smaller than those of the younger larvae and were free from the pigment.

Most of the larvae were found near the margins of the retina although some were at other levels including that portion immediately behind the lens. Consequently, it is probable that even moderately heavy infections in perch do not seriously impair vision. Experimentally infected perch harboring large numbers of worms did not seem to be adversely affected.

RELATIONSHIPS

C. emarginatae and its metacercaria are strikingly similar morpho-

logically to the corresponding stages in the life cycle of *Diplostomum flexicaudum*. These similarities must indicate that the two species are closely related and it seems reasonable to predict that the adult of *C. emarginatae* is a member of the DIPLOSTOMIDAE and is probably in the genus *Diplostomum*.

SUMMARY

Infection experiments have demonstrated that the cercaria described by Van Haitsma (1930) as the cercaria of *Cotylurus communis* does not belong to that species. These cercariae, for which the name *Cercaria emarginatae* Cort, 1917 is brought forward, penetrate perch (*Perca flavescens*) and develop in the eyes to typical, unencysted diplostomula similar to those of *Diplostomum flexicaudum*. The metacercariae of *C. communis* are known to be encysted tetracotyle larvae which occur principally about the hearts of fishes. Additions are made to the description of *C. emarginatae*, and the metacercariae into which it develops are described. The metacercariae develop almost exclusively in the retina between the pigmented layer and the layer of sensory cells. When the larvae are young they evidently feed on the tissue of the retina since they contain large quantities of pigment. Since most of the larvae occur in the peripheral portions of the retina and do not cause extensive damage, they probably do not interfere seriously with vision unless present in very large numbers.

BIBLIOGRAPHY

- CORT, W. W. 1917 Homologies of the excretory system of the forked-tailed cercariae. *J. Parasitol.* 4: 49-57.
- CORT, W. W. AND BRACKETT, S. 1937 Two new species of strigeid cercariae from the Douglas Lake region, Michigan. *J. Parasitol.* 23: 265-280.
- AND ——— 1938 A new strigeid cercaria which produces a bloat disease in tadpoles. *J. Parasitol.* 24: 263-271.
- DUBOIS, G. 1938 Monographie des Strigeida (Trematoda). *Mém. Soc. Neuchâtel. Sc. Nat.* 6: 1-535.
- HUGHES, R. C. 1928 Studies on the trematode family Strigeidae (Holostomidae). No. XIII. Three species of tetracotyle. *Tr. Am. Micr. Soc.* 47: 414-433.
- HUGHES, R. C. AND BERKHOUT, P. G. 1929 Studies on the trematode family Strigeidae (Holostomidae). No. XV. *Diplostomulum gigas* sp. nov. *Papers Michigan Acad. Sc., Arts, and Letters* 10: 483-488.
- LA RUE, G. R. 1932 Morphology of *Cotylurus communis* Hughes (Trematoda: Strigeidae). *Tr. Am. Micr. Soc.* 51: 28-47.
- OLIVIER, L. 1940 Life history studies on two strigeid trematodes of the Douglas Lake region, Michigan. *J. Parasitol.* 26: 447-477.
- SZIDAT, L. 1931 Beiträge zur Entwicklungsgeschichte der Holostomiden. IV. Die Cercarie des Entenparasiten *Apatemon (Strigea) gracilis* Rud. und ihre Entwicklung im Blutgefäßssystem des Zwischenwirtes (*Herpobdella atomaria* Car.). *Z. Parasitenk.* 3: 160-172.
- VAN HAITSMAN, J. P. 1930 Studies on the trematode family Strigeidae (Holostomidae). XXI. Life cycle and description of the cercaria of *Cotylurus michiganensis* (LaRue). *J. Parasitol.* 16: 224-230.

ON TWO NEW SPECIES OF MYXOSPORIDIA FROM ILLINOIS FISHES*

PAUL A. MEGLITSCH

Wright Junior College, Chicago

During the spring and summer of 1935, a number of Illinois fishes were autopsied. In a previous publication (1937) the writer presented a summary of the Myxosporidia found during the study, including the record of a number of forms identified to genus but not speciated. Two of these were restudied and are described in this paper.

All of the fishes were taken in the Ohio River and shipped to Urbana, Illinois, on ice. The material was studied as rapidly as possible in fresh condition. In most cases the Myxosporidia were still alive and motile during the preliminary observations. The parasites were preserved in 4% formaldehyde and have been studied recently in stained and unstained preparations. Permanent slides were stained with Heidenhain's iron haematoxylin or Giemsa.

Chloromyxum opladeli n. sp.

(Figs. 1-9)

Habitat. This myxosporidian occurs in the gall bladder of *Opladelus olivaris*. Two of twenty fishes examined were infected, the gall bladder of one of which also contained *Myxidium* sp. These fishes were obtained from the Ohio River at Shawneetown and Rosiclair, Illinois.

Trophic stage. One host contained large numbers of active trophozoites; the other contained many mature spores but few vegetative forms. Immature trophozoites were small, with about 10 μ the minimum diameter. Mature forms with developing spores measured from 18 to 30 μ in diameter. The hyaline ectoplasm formed a broad girdle about the oval, somewhat flattened organism. The granular endoplasm contained many small refractive inclusions. Many nuclei, visible in vivo, stained deeply with basic dyes. Developing pansporoblasts and mature spores were found in the alveolar endoplasm.

Spore. The majority of spores were typical of the genus *Chloromyxum*. They were subspherical in front and side views and contained four polar capsules at the anterior end of the spore. A rather prominent, slightly elevated, sutural ridge, visible in both fresh and stained spores, was present (Figs. 2, 3). In some cases the sutural ridge was

Received for publication, March 29, 1941.

*The writer wishes to acknowledge his indebtedness to Dr. D. H. Thompson of the Illinois Natural History Survey, who supplied and identified the fishes; and to Prof. R. R. Kudo of the University of Illinois for his helpful criticism of the manuscript.

slightly curved. Each valve bore up to eleven distinct striae which converged anteriorly. The sporoplasm was small and filled the extracapsular cavity of the spore. Two small nuclei were found, occupying a somewhat excentric position in the majority of spores (Fig. 4). The polar capsules were pyriform, converging slightly, but opening separately. Small, flattened capsulogenous nuclei were persistent, and could be seen in a pycnotic condition in most of the stained spores. The polar capsules were approximately equal in size. Spore dimensions were: length, 6.5–8 (7.4) μ ; breadth, 6–7.5 (6.7) μ ; thickness, 5.5–7 (6.3) μ ; capsules 2.5–3 (2.8) μ by 1.5–2.3 (1.9) μ .

Atypical spores, with two, six or eight polar capsules, were quite numerous. In the two hosts those with six polar capsules comprised 30% and 25% of all spores present. In their major morphological characters they resembled the typical spores, but were somewhat larger, and the polar capsules were somewhat smaller. There were three shell valves, fitting together along three distinct sutural ridges (Fig. 5). In anterior view the spore had a rather triangular shape. Striations similar to those found on typical spores ran more or less parallel to the sutural ridges. The polar capsules were pyriform and relatively small. Each bore a small flattened, pycnotic capsulogenous nucleus (Fig. 6). The sporoplasm was large and contained three nuclei. These spores averaged about 8.3 μ in length; 7.5 μ in breadth; 7.4 μ in thickness; capsules 2.5 by 1.6 μ .

Relatively small numbers of spores with two polar capsules occurred. 5% and 3% of all spores were of this type in the two hosts. These spores were smaller than typical spores but had few distinctive characteristics. The spore membrane was thin, and there was a slightly elevated sutural ridge (Fig. 6). Seven to nine striae were present on each valve, converging anteriorly. The average dimensions of the few spores of this type were: length, 6.3 μ ; breadth, 6.0 μ ; thickness, 5.8 μ ; capsules 2.5 by 1.9 μ .

Occasionally spores with eight polar capsules were found. The morphology of these is not well known because of the small numbers in which they occurred (about 1% of all spores in each host). The spore membrane was composed of four valves meeting along low sutural ridges (Fig. 9). Striae, converging anteriorly, were present on each valve. A typical spore of this type measured: length, 8.4 μ ; breadth, 7.6 μ ; capsules, 2.5 by 1.2 μ .

Discussion. Atypical spores are not rare among Myxosporidia. They have been described in a number of species belonging to several genera and probably are more widespread than the literature might suggest as they usually occur in relatively small numbers and present no important morphological problem. Aberrations of various types are

known. One of the commonest forms of aberrancy is the appearance of short caudal processes on spores of *Myxobolus* or *Myxosoma*, as, for example, *Myxobolus hylae* (Johnston and Bancroft, 1918), *Myxobolus mulleri* (Bütschli, 1881), *Myxosoma dujardini* (Thélohan, 1895) and *Myxosoma funduli* (Kudo, 1918). A change in the size or position of polar capsules may produce abnormal asymmetrical spores, as in *Myxobolus aeglefinis* (Auerbach, 1906), *Myxobolus orbiculatus* (Kudo, 1920), and *Mitraspora elongata* (Kudo, 1920). An aberration involving the appearance of a supernumerary polar capsule occurs in *Chloromyxum catostomi* (Kudo, 1920). Although such atypical spores have an altered morphology there is little effect on the major axes of the spore. The writer is familiar with but few instances in which the aberrancy involves a readjustment of the axial arrangement of the spore. Noble (1939) mentions and figures spores with four polar capsules which he found frequently in fishes infected with *Trilospora californica*. The spores are normally characterized by tri-radiate symmetry but spores with four capsules are tetra-axiate. This is especially interesting since the typical spores resemble a tri-radiate *Ceratomyxa*.

The present form is unusual, then, in having such a high frequency of aberrant spores, and in the extent to which the aberrancy effects the axis of the spore. The bilateral symmetry of the typical *Chloromyxum* spore is supplanted by tri-radiate symmetry in spores with six polar capsules, and tetra-radiate symmetry in spores with eight polar capsules. Whether such aberrancies represent a response on the part of the parasite to an unfamiliar host, similar to that observed by Bond (1937) in *Myxosoma grandis*, or are the result of some internal factor, is not yet known.

The mode of formation of aberrant spores presents several interesting problems. Any one trophozoite may produce several kinds of spores. Fig. 1 shows a trophozoite containing one spore with eight polar capsules and two lying together with two and six polar capsules. Each pansporoblast must give rise to four sporoplasmic nuclei, four nuclei associated with the spore membrane and eight capsulogenous nuclei, distributed equally to the two spores formed, if development is typical. In many myxosporidia two residual nuclei remain in the pansporoblast. In the formation of atypical spores the distribution of nuclei to the spores is modified. A pansporoblast can give rise to one spore with eight polar capsules or two spores with two and six polar capsules respectively. In the spore with eight polar capsules, all of the nuclei are given to the single spore. Each polar capsule has one capsulogenous nucleus. The sporoplasm in some spores studied carried four nuclei. Unfortunately, so few developing spores of this type have been found that it is not possible to determine whether the usual relationships of valves to shell nuclei prevail, but it

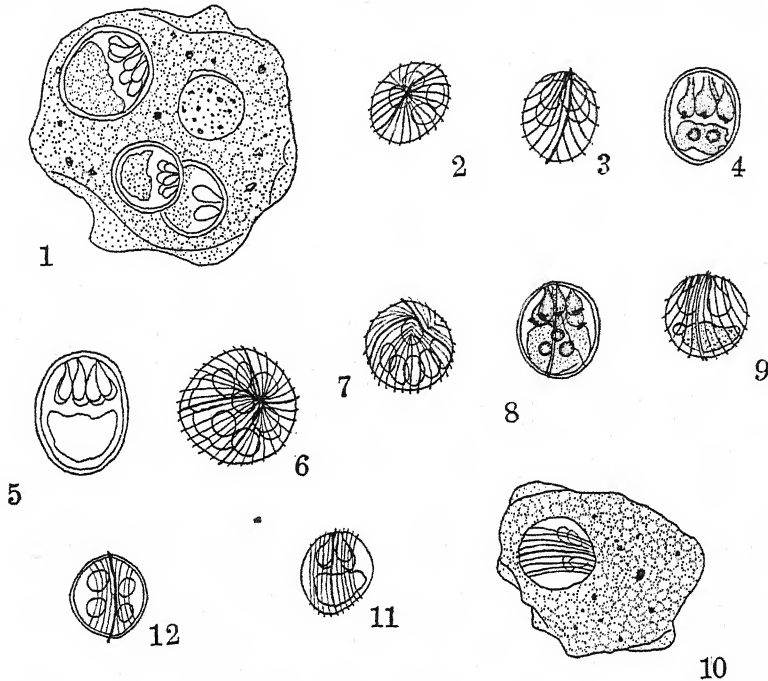
seems probable that the four valves are each accompanied by a single nucleus. Thus all of the nuclei of the pansporoblast are accounted for.

Spores with two polar capsules and six polar capsules developing from a pansporoblast present a discrepancy in the number of nuclei. The eight capsulogenous nuclei are accounted for, six being given to one spore, two to the other. The sporoplasmic nuclei are likewise accounted for since there are three in the spore with six polar capsules and one in the spore with two polar capsules. In nuclei associated with the spore membrane, however, there is a discrepancy. In typical development, four such nuclei are formed, two going to each spore, which, in consequence is bivalve. But in this type of atypical development the six-capsuled spore has three valves and the bicapsular spore has two valves. Thus five valves develop from the pansporoblast. Preliminary observations have shown that there are three shell nuclei in the developing spores with six polar capsules, and several spores with two polar capsules have been found which contained two shell nuclei, one associated with each valve. This extra nucleus cannot come from capsulogenous nor sporoplasmic nuclei since these are all accounted for. Thus one of the valve nuclei may undergo an extra division, or one of the residual nuclei of the pansporoblast may be used. It has not been determined which of these takes place.

According to the observations made to the present time the spores with six polar capsules develop from a pansporoblast which gives rise to another spore with two polar capsules. The two types of spores are seen lying side by side in trophozoites not infrequently (Fig. 1). When floating free in the bile, however, a high percentage of spores with six polar capsules are found (25-30%) and a very low percentage with two polar capsules (3-5%). If the two develop together there should be an equal number of each. Although a few sporoblasts developing into single typical spores have been seen, none developing into spores with six polar capsules have been found. Even though some may develop in this way, it cannot account for the whole discrepancy since they must be quite rare. The inference is drawn that spores with two capsules rarely complete their development or degenerate in the bile soon after their formation.

Identification. Of the 41 species of *Chloromyxum* which are listed in Kudo (1933), none have characteristics which can be considered identical with the present form. Of the various species described by various investigators those most closely resembling the present form, exclusive of the formation of atypical spores, are *Chloromyxum fluviatile* Thélohan, *Chloromyxum truttae* Légèr, *Chloromyxum thymalli* Lebzelter, *Chloromyxum misgurni* Kudo, and *Chloromyxum granulosum* Davis. *Chloromyxum fluviatile* is differentiated by the posterior points

shown on the shell valves (Thélohan, 1892 discussed by Kudo, 1920). *Chloromyxum truttiae* has a slightly different spore shape and has trophozoites either elongate or without pseudopodia (Légèr, 1906).



Figs. 1-12

All figures are camera lucida drawings magnified 1590 times. Unless otherwise stated the spores are preserved in 10% formalin and unstained.

Chloromyxum opladeli

1. Trophozoite with atypical spores.
2. Typical spore, oblique view.
3. Typical spore, side view.
4. Typical spore, front view. Heidenhain's haematoxylin.
5. Spore with six polar capsules.
6. Spore with six polar capsules. Heidenhain's haematoxylin.
7. Spore with two polar capsules, side view.
8. Spore with eight polar capsules.
9. Spore with eight polar capsules.

Chloromyxum thompsoni

10. Trophozoite containing a young spore.
11. Spore, side view.
12. Spore, anterior view.

Chloromyxum thymalli may be distinguished from the present form by its tendency to produce six spores in each trophozoite, and the somewhat wavy valve striations (Lebzelter, 1912). *Chloromyxum misgurni*

differs from the present form by its habit of attaching to the wall of the gall bladder in the trophic stage, the failure of the sutural lines to converge anteriorly, and the straight sutural line (Kudo, 1916). *Chloromyxum granulorum* is differentiated by the small number of striations on the spore valves (Davis, 1917). As a result, this form has been considered as a new species, and the name *Chloromyxum opladeli*, is proposed for it.

Chloromyxum thompsoni n. sp.

(Figs. 10-12)

Habitat. This organism occurs in the gall bladder of one *Ictiobus bubalus* of three examined. All of the fishes were from the Ohio River at Shawneetown, Illinois.

Vegetative form. A few trophozoites bearing spores were found. These were small amoeboid forms measuring from 15 to 25 μ in diameter (Fig. 10). There was no distinct ectoplasm and movement was very sluggish. Probably the trophic stages were somewhat abnormal when first examined. In stained trophozoites many small nuclei were found in the granular, alveolated endoplasm. Pansporoblasts and developing spores were numerous. The trophozoites are usually polysporous, containing from two to four pansporoblasts in different stages of development.

Spore. The spore was small and subspherical, with rather thick valves and a conspicuous sutural ridge. Each valve bore about five well developed, distinctly elevated striations which extended from the anterior to the posterior end of the spore. They lay parallel to the sutural ridge, and covered the median half of the spore membrane (Figs. 11, 12) in side view. The faces of the valves carried no striae. Four large, broadly pyriform polar capsules occupied the anterior half of the spore. The capsulogenous nuclei were not found in mature spores. Valve nuclei were also lost before the spore became mature. Two small sporoplasmic nuclei lay in the sporoplasm which occupied the posterior half of the spore cavity. The sporoplasm was usually indented at the base of the capsules. Spores measured approximately 6 to 8 (6.9) μ in length, 6 to 7.5 (6.5) μ in breadth, 5.5 to 7 (6.3) μ in thickness. Capsules measured 2 to 3 (2.6) μ by 1.5 to 2 (1.6) μ .

Identification. The species which most closely resemble the form under consideration are *Chloromyxum fluviatile* Thélohan, *C. granulorum* Davis and *C. catostomi* Kudo. *C. fluviatile* differs from the present form by having three or four fine points on the posterior half of the spore (Thélohan, 1892, discussed by Kudo, 1920). *C. granulorum* differs in having striae which converge anteriorly (Davis, 1917). *C. catostomi* is most similar, but differs in having trophozoites with filiform pseudopodia attached to the wall of the gall bladder, more rounded polar

capsules, and very fine striations instead of relatively coarse ones as found in the present form (Kudo, 1920). No atypical spores with five capsules similar to those described in *Chloromyxum catostomi* have been found. For these reasons the present form is believed to be an undescribed species, and the name *Chloromyxum thompsoni* is proposed for it.

SUMMARY

1. Two new species of *Chloromyxum* from Illinois fishes are described, *Chloromyxum opladeli* n. sp. from the gall bladder of *Opladelus olivaris* and *Chloromyxum thompsoni* n. sp. from the gall bladder of *Ictiobus bubalus*.

2. Atypical spore formation is recorded in *Chloromyxum opladeli*, and briefly discussed.

BIBLIOGRAPHY

- AUERBACH, M. 1906 Weitere Mittheilung über *Myxobolus aeglefini*. Zool. Anz. 31: 115-119.
- BOND, F. F. 1937 *Myxosoma grandis* Kudo in fish from the Hudson River drainage system. J. Parasitol. 23: 231-232.
- BÜTSCHLI, O. 1881 Beiträge zur Kenntniss der Fischsporospermien. Z. Wissensch. Zool. 35: 629-651.
- DAVIS, H. S. 1917 The Myxosporidia of the Beaufort region. Bull. U. S. Bureau Fish. 35: 201-243.
- KUDO, R. R. 1916 Contributions to the study of parasitic protozoa. III. Notes on some Myxosporidia found in some fresh-water fishes of Japan, with the description of three new species. J. Parasitol. 3: 3-9.
- 1918 Contributions to the study of parasitic protozoa. IV. Note on some Myxosporidia from certain fish in the vicinity of Woods Hole. J. Parasitol. 5: 11-16.
- 1920 Studies on Myxosporidia. A synopsis of genera and species of Myxosporidia. Illinois Biol. Monogr. 5 (Nos. 3 and 4): 1-265.
- 1933 A taxonomic consideration of Myxosporidia. Tr. Am. Micr. Soc. 52: 195-216.
- LEBZELTER, V. 1912 Ueber Protozoen aus der Gallenblase von *Thymallus thymallus* L. Zool. Anz. 50: 295-297.
- LÉGER, L. 1906 Myxosporidies nouvelles parasites des poissons. I. Sur une nouvelle maladie myxosporidienne de la truite indigne. II. Sur une nouvelle Myxosporidie de la tanche commune. Ann. Univ. Grenoble 18: 267-272.
- MEGLITSCH, P. 1937 On some new and known Myxosporidia of the fishes of Illinois. J. Parasitol. 23: 467-477.
- NOBLE, E. R. 1939 Myxosporidia from the tide pool fishes of California. J. Parasitol. 25: 359-364.
- THÉLOHAN, P. 1895 Recherches sur les Myxosporidies. Bull. Scient. France et Belgique 26: 100-394.

RESEARCH NOTES

A FURTHER NOTE ON LIFE HISTORY EXPERIMENTS WITH *CRYPTOCOTYLE LINGUA* (CREPLIN, 1825)

During the last five years a series of life history studies on heterophyid trematodes have proved unsuccessful owing to the fact that various animals used as final hosts have again and again proved immune to infection.

In the case of *Cryptocotyle lingua* the flukes had failed to develop in laboratory reared white rats, domestic ducks and black-headed gulls, *Larus ridibundus* L. (Rothschild, 1939, Novitat. Zool. Tring, 41: 178-180), although successful infections had eventually been obtained in two gulls which had been deprived of vitamins in their diet. The long series of worms thus obtained all displayed low egg production. The maximum number of eggs in any fluke was about 30, and the egg shells themselves were very thin. It was suggested at the time that this limited egg production might be accounted for by the fact that the black-headed gull was not a natural host for this species of trematode.

A different explanation, however, seemed worth testing, namely that the metacercariae obtained from certain laboratory infected fish were neither as robust nor as viable as specimens obtained from the same species of fish under natural conditions.

Two gulls were accordingly fed gobies, *Gobius ruthensparri* (Euphras.), which had acquired their infections in the wild. The results obtained certainly seem to confirm this hypothesis.

Eggs began to appear in the feces six days after feeding cysts. When the flukes were recovered from the intestines of the bird, when they were killed three weeks later, almost all proved to be larger than the largest of the specimens obtained from the gulls fed with experimentally infected fish. Moreover, the number of eggs per fluke was far greater than that found in the specimens from the original experiments. Measurements and egg counts of five preserved specimens chosen at random from each group are given below.

Adult worms obtained after feeding gulls with gobies infected in the wild		Adult worms obtained after feeding gulls with gobies infected in the laboratory	
Size in microns (maximum length and width)	No. of eggs per specimen	Size in microns (maximum length and width)	No. of eggs per specimen
1925 × 495	145	1530 × 525	6
1650 × 525	36	1320 × 525	12
1305 × 555	191	1140 × 360	24
1275 × 646	222	1050 × 420	15
1200 × 660	89	930 × 345	13
Mean: 1471 × 576	137	1194 × 435	14

The most striking feature, however, is the relatively thicker egg-shell found in the first group—the eggs being consequently a rich dark brown in color instead of a deep yellow. This gives a false impression of greater size.

The metacercariae, when removed from the cysts of naturally infected gobies, do not appear to differ morphologically from those which develop in experimentally infected fish. Some specimens, however, are very active, moving about vigorously between slide and cover glass. In these specimens the various organs seem more sharply differentiated, and the cyst wall is more resistant. This active type was not found in the artificially infected gobies, which were all of the sluggish variety, barely showing more than feeble contractions when released from the cyst. On the other hand, in naturally infected fish I have found both sluggish and active metacercariae measuring approximately the same size in the same individual fish in a similar location along the fin rays. On the whole it seems reasonable to suppose that, although apparently quite healthy in the aquarium, few if any fish enjoy such perfect health in captivity as they do in natural conditions. As in the case of cer-

tain snails and their cercariae (Rothschild, 1938, Novitat. Zool. Tring, 41: 84-102), this poor condition of the host is duly reflected in the lack of vitality and general physiological state of the parasites. This might well account for the frequent failure of the metacercariae to develop and establish themselves in an apparently suitable final host in the laboratory. Whatever the fundamental cause may be, the adult flukes, which were obtained from black-headed gulls fed with naturally infected gobies, are unquestionably more normal specimens and agree more closely with published descriptions of *C. lingua* than those recovered after feeding with metacercariae obtained experimentally.—MIRIAM ROTHSCILD, *Ashton, Peterborough, England.*

NEW PARASITE RECORDS FROM THE RUFFED GROUSE

In the course of examining a ruffed grouse (*Bonasa umbellus*) from Itasca Park, Minnesota, on August 19, 1940, the author found two species of trematodes hitherto unrecorded from this host.

Two specimens of *Lyperosomum monenteron* Price and McIntosh (1935, Proc. Helm. Soc. Washington 2: 63-64) were collected in the liver. The specimens agree with the figure in the original description in general appearance and in the peculiar condition of having only one cecum. Measurements of the present specimens in millimeters are given below with the measurements from the original description inserted in parentheses for comparison: body 4.9 to 5.3 (1.9 to 5.2) long; oral sucker 0.234 to 0.277 (0.120 to 0.170) long by 0.213 to 0.284 (0.123 to 0.150) wide; acetabulum 0.213 to 0.284 (0.170 to 0.320) long by 0.249 (0.200 to 0.320) wide; pharynx 0.064 to 0.072 (0.042 to 0.060) long to 0.056 (0.050 to 0.070) wide; ova 0.032 to 0.038 (0.032) long by 0.019 to 0.027 (0.016) wide. The same species was found in the livers of two kingbirds (*Tyrannus tyrannus*) from the same locality.

One specimen of *Echinoparyphium aconiatum* Dietz (1910, Zool. Jahrb. Jena Suppl. 12: 265-504) was found in the intestine. This species is distinguished from other 37-spined echinostomes by the location of the ventral sucker one-fourth of the body length from the oral sucker and by the shortness of the uterus. The present specimen agrees closely in size with those of Dietz as will be seen in the following measurements given in millimeters. Measurements from the original description are inserted in parentheses for comparison. Body 1.53 (1.62) long by 0.35 (0.26) wide; long spines 0.040 (0.033 to 0.048) long by 0.0112 (0.006 to 0.009) wide; short spines 0.027 (0.028 to 0.045) long by 0.008 (0.0048 to 0.0072) wide; oral sucker 0.085 (0.07 to 0.083) wide; pharynx 0.085 (0.065) long; acetabulum 0.241 (0.22) long; ova 0.1008 to 0.1072 (0.1008 to 0.1032) long by 0.0592 to 0.688 (0.066 to 0.068) wide. Riech (1927, Centr. Bakt. I Abt. Orig. 103: 279-290) found that cercariae of this species encysted in several species of snails.

The author is indebted to Dr. F. G. Wallace for suggestions in preparing this note.—NOBUTARO ISHII, *Lake Itasca Forestry and Biological Station, University of Minnesota.*

A DEVICE FOR ADMINISTERING PARTICULATE MATERIAL TO MICE

In administering parasite cysts or larvae by stomach tube to mice and other small animals one encounters certain difficulties in expelling all of the cysts from either the rubber bulb pipette or the Luer syringe. The simple pump described here was devised in order to provide a dependable means of injecting definite numbers of trematode cysts through a stomach tube. With this pump it is possible to follow the dose of infective material with a "chaser" of water which washes out the tube and both hands are left free for the manipulation of the animal and stomach tube.

A bottle of water with a two-hole rubber stopper is fitted with a delivery tube and an air inlet tube (Fig. 1). The delivery tube is continued by a rubber tube one foot long to the stomach tube which may be of glass or of cellophane (Spur-

lock, 1941, J. Parasitol. 27: 93). We have found that a piece of surgical stitch tubing cut obliquely at the end is very satisfactory. A small bulb in the glass connection to the stomach tube keeps the cysts concentrated in one place. Midway between the bottle and the stomach tube a rubber tube with the end plugged and long enough to reach the floor is connected to the delivery tube by a glass T. Two small Bunsen valves are inserted, one on either side of the T. The Bunsen valve (Fig. 2) is made by fitting a one-inch piece of a No. 10 soft rubber catheter on the

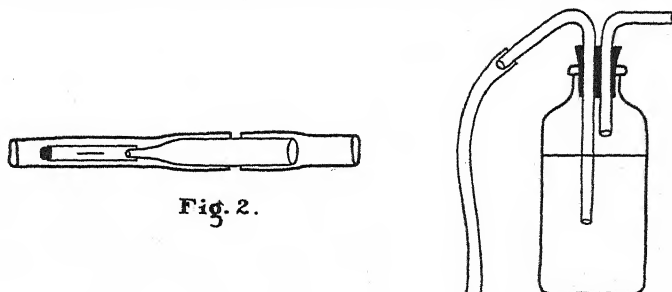


Fig. 2.

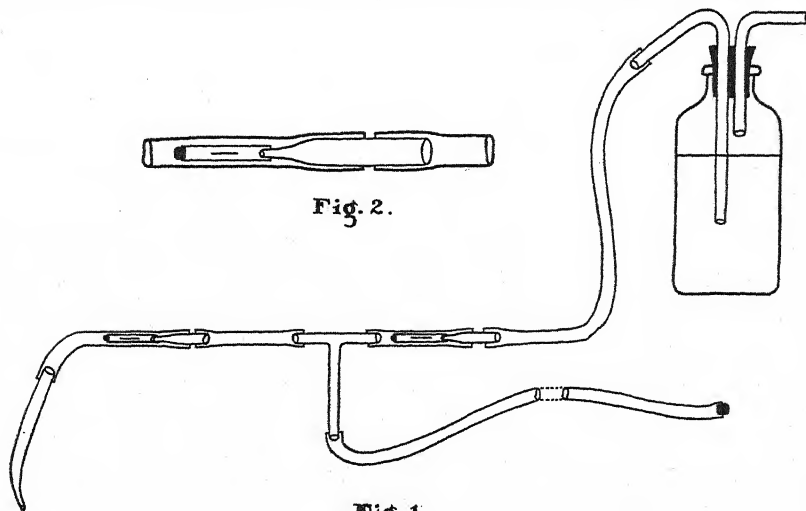


Fig. 1.

tapered end of a piece of glass tubing. The end is plugged and a longitudinal slit is cut with a razor blade. When the entire system is filled with water and all air bubbles are expelled it forms a miniature pump and when one steps on the tube on the floor a few drops of water are ejected from the end. The rubber tube to the left of the second valve is used as a pipette in order to suck up the cysts. Once in the bulb the cysts are locked in place by the valve until pressure is applied to the pump.—F. G. WALLACE, *Department of Zoology, University of Minnesota*.

THE DURATION OF HUMAN INFECTION WITH *ENDAMOEBIA HISTOLYTICA* AND OTHER INTESTINAL PROTOZOA

A few students in the Vanderbilt University School of Medicine have been observed over a period of years with regard to their intestinal protozoa. Students harboring *Endamoeba histolytica*, who had had no symptoms referable to the infection, were requested not to obtain treatment, so that the duration of their infection could be determined. Two stool examinations were performed in October of the first academic year, another during May of the second year, and thereafter at irregular intervals. The following report concerns nine students who harbored *E. histolytica*.

In two students observed for four and a half years, stool specimens were positive each time they were examined. In two observed for three and a half years and in three observed for one and a half years, the same results were obtained. Of the other two students, one was positive on the first examination and negative on the second examination one and a half years later and continued to be negative up to four and a half years. The other was positive on the first examination and negative on the second examination one and a half years later. He was not observed thereafter.

Complement fixation tests were performed two or more times on seven of these students in our laboratory, using an alcoholic extract of washed motile amoebae as antigen, and an antish sheep hemolytic system. All were negative. Colonel Charles F. Craig performed one complement fixation test on six of these, using an anti-human hemolytic system, and obtained +++ reactions in two, +++ reactions in two, and \pm reactions in two. One of Colonel Craig's +++ reactions was obtained in a student whose stool specimen had been negative for *E. histolytica* on several occasions for at least two months before the blood was drawn for the test. None of these students developed symptoms referable to amoebiasis during the period of observation.

Observations of other intestinal protozoa in the same students were as follows: One student observed for four and a half years showed *E. coli*, *E. nana* and *Iodamoeba* on the first examination and one and a half years later, and *Iodamoeba* after four and a half years. The student who was observed for four and a half years but showed *E. histolytica* only on the first examination also showed *E. coli* and *E. nana* on this examination but not on subsequent examinations. One student observed for three and a half years showed *E. coli* only on the first examination, but showed *Giardia* on each examination. One student observed for one and a half years showed *E. coli* throughout this period, and *E. nana* was found at the end of one and a half years but not on the original examinations. Another student observed for one and a half years showed *E. coli* throughout this period.

Although this series is small, it is recorded at this time because of the impossibility of further observations.—HENRY E. MELENEY, *Vanderbilt University School of Medicine, Nashville, Tennessee.*

SIGMODON HISPIDUS HISPIDUS, A NEW HOST FOR THE STROBILOCERCUS OF TAENIA TAENIAEFORMIS

We recently received at this laboratory a male cotton rat, *Sigmodon hispidus hispidus*, from the Michigan Department of Health. This animal was from their breeding colony maintained for investigations on poliomyelitis. It was one of the original stock, having been trapped in South Carolina in February, 1940. The rat had been partially eaten, but examination of the liver revealed the presence of eleven strobilocerci of *Taenia taeniaeformis*. They were from 7 to 10 mm in diameter fixed. The larvae removed from the cysts were from 84 to 135 mm in length with a mean of 114 mm. There were from 32 to 38 hooklets on the rostellum, the large hooklets averaging 316 microns in length and the small hooklets 238 microns.

To the best of our knowledge this is the first record of the strobilocercus of *Taenia taeniaeformis* from the cotton rat.—PHILIP A. HAWKINS, *Department of Bacteriology and Hygiene, Michigan State College.*

Dates for mailing of numbers of volume 27 (1941):

No. 1, February 10.

No. 2, April 10.

No. 3, June 10.

No. 4, August 8.

No. 5, October 10.

No. 6 (with December Supplement), December 10.

The Journal of Parasitology

Volume 28

APRIL, 1942

Number 2

THE BUOYANCY OF CERTAIN NEMATODE EGGS*

WILLI SAWITZ

Department of Tropical Medicine, Tulane University of Louisiana,
New Orleans, Louisiana

Although floatation technics for the recovery of eggs of parasites from fecal specimens make use of the buoyant force exerted by solutions of a specific gravity higher than that of the eggs, specific gravity values have been reported only for eggs of *Ascaris lumbricoides* (1) and of *Necator americanus* (2). The present study deals with the eggs of the human pinworm (*Enterobius vermicularis*), the human whipworm (*Trichuris trichiura*), the dog whipworm (*Trichuris vulpis*), the dog hookworm (*Ancylostoma caninum*) and fertilized and unfertilized eggs of *Ascaris lumbricoides* from man.

METHODS

Fecal specimens containing eggs of *Enterobius vermicularis*, *Trichuris trichiura* and *Ascaris lumbricoides* were brought to the laboratory of this Department for routine examination. Feces containing eggs of *Trichuris vulpis* and *Ancylostoma caninum* were passed by naturally infected laboratory dogs.

The fecal samples containing the eggs were thoroughly homogenized in physiologic salt solution (approximately 1:50) by a stirring machine (3), which was allowed to run for one hour at 1,600 RPM. The homogenized suspension was strained through one layer of cheesecloth into a gallon jar, which was then filled with physiologic salt solution. Sedimentation was allowed to take place for 6 to 12 hours, usually overnight, following which the supernatant liquid was siphoned off and the sediment containing the concentrated egg suspension kept in the refrigerator at 5° C. The approximate number of eggs present in a measured amount of

Received for publication, April 7, 1941.

* This communication is one of a series entitled "A Critical Study of Clinical Laboratory Technics for the Diagnosis of Protozoa and Helminths in Feces," by the Tulane Amebiasis Unit of the National Institute of Health in collaboration with the Department of Tropical Medicine at Tulane University, under the direction of Ernest Carroll Faust.

Aided by a grant from the David Trautman Schwartz Research Fund of Tulane University.

suspension was determined by the centrifugal floatation technic (3), using a 45.6 per cent zinc sulphate solution ($\text{ZnSO}_4 + 7\text{H}_2\text{O}$ U.S.P. granular) of specific gravity 1.250 in a 10 ml Wassermann tube (100×13 mm) and a coverglass resting snugly on the ground top of the tube.

The specific gravities of the eggs were tested by centrifugal floatation in zinc sulphate solutions of graded specific gravities. Equal amounts of suspension containing a known number of eggs were pipetted into each of several Wassermann tubes with ground top. Zinc sulphate solutions of the various specific gravities were then added to fill the tubes so that a superimposed round coverglass contacted the liquid except for a small area occupied by an air bubble. This provided a safeguard against spilling of some of the liquid together with eggs. The preparations were centrifugalized in metal holders with metal sleeves having four fingers projecting upwards to hold the coverglasses in place. An International clinical centrifuge was used with an acceleration period of 20 seconds, a top speed of 2,640 RPM and a deceleration period of 30 seconds. The distance from the axis of the centrifuge to the bottom of the tube was 13.5 cm. After centrifugation for 100 seconds at top speed the coverglasses were rapidly but carefully lifted off each tube, gently rotated manually to mix the material uniformly throughout the adhering drop, and placed drop-down on a vaseline-ringed slide. To the tube from which the coverglass was removed there was added its appropriate zinc sulphate solution until the meniscus reached the rim and a new coverglass was applied. This procedure was repeated again, thus providing 3rd, 4th, and 5th coverglass preparations.

The supernatant liquid remaining after the last preparation had been made was approximately halved by pipetting the upper and lower portions into separate tubes. Zinc sulphate solution of specific gravity 1.250 or 1.300 was added to be sure that the resulting solution exceeded a specific gravity of 1.200. Two to three coverglass preparations were made from each tube.

To the sediment of the original tubes zinc sulphate solution of specific gravity 1.250 was added, the sediment broken up by shaking, and 5 to 7 coverglass preparations were made.

Finally, the sediment of each tube was examined directly by spreading the material on slides to detect eggs which failed to float. The number so found was always less than 1 per cent.

The number of eggs on each coverglass preparation was counted and tabulated. The percentages of recovery from each layer were calculated on the basis of the total number of eggs recovered from each tube. The results show the distribution of the eggs in the surface layer, upper and lower portions of the supernatant liquid, and in the sediment, after centrifugation in zinc sulphate solutions of various specific gravities.

RESULTS

The data in each series of experiments are presented as the percentages of the total number of eggs tested in each solution.

Eggs of the pinworm (*Enterobius vermicularis*).—The specific gravity of pinworm eggs was tested in 3 experiments. In the first two experiments 0.5 ml of the suspension was used, each containing approximately 70 eggs, in the third experiment 0.5 ml containing approximately 90 eggs. The summarized results are presented in Table 1.

TABLE 1.—Percentages of recovery of pinworm eggs from different levels of $ZnSO_4$ solutions of various specific gravities. Summary of 3 experiments

Specific gravity	1.080	1.100	1.110	1.115	1.120	1.125	1.180
Surface layer	2	2	6	57	71	85	97
Supernatant { upper	0	0	1	6	4	2	0
{ lower	2	2	2	1	1	2	1
Sediment	96	96	91	36	24	11	2

No essential difference was found when fully embryonated pinworm eggs were tested 24 hours after being passed and after 5 days in the refrigerator.

The greater the yield of eggs is on the first coverglass preparation removed from the surface layer, the more efficient is the concentration by centrifugal floatation. For *Enterobius vermicularis* eggs the efficiency percentages of the first coverglass preparations removed from the solutions of the various specific gravities were as follows: specific gravity 1.080, 2; 1.100, 2; 1.110, 1; 1.115, 12; 1.120, 56; 1.125, 44 and 1.180, 70.

Whipworm eggs.—The specific gravity of eggs of the human whipworm (*Trichuris trichiura*) was tested in 5 experiments with no essential difference in the results. The amounts of suspension used were 0.2 ml containing approximately 150 eggs, 0.2 ml with approximately 200 eggs, 0.4 ml with approximately 210 eggs, 0.4 ml with 150 eggs and 1 ml with approximately 300 eggs. The summarized data are presented in Table 2.

TABLE 2.—Percentages of recovery of *Trichuris trichiura* eggs from different levels of $ZnSO_4$ solutions of various specific gravities. Summary of 5 experiments

Specific gravity	1.120	1.130	1.140	1.150	1.160	1.165	1.170	1.180	1.200	1.300
Surface layer	0	1	2	23	49	66	75	85	99	100
Supernatant { upper	0	0	2	7	5	5	2	5	0	0
{ lower	0	0	4	7	7	5	4	3	0	0
Sediment	100	99	92	63	39	24	19	7	1	0

The efficiency percentages of the first coverglass preparations removed from the surface layers of the various solutions were as follows: specific gravities 1.120 and 1.130, 0; 1.140, 1; 1.150, 8; 1.160, 33; 1.165, 26; 1.170, 32; 1.180, 52; 1.200, 90; and 1.300, 93.

Centrifugation times of 50 and 280 seconds at top speed yielded fewer eggs than did 100 seconds. By employing sodium chloride solutions of specific gravities 1.180 and 1.200 the percentages of eggs buoyed up were

respectively 18 and 73 as compared to 85 and 99 in zinc sulphate solutions of the same specific gravities. The corresponding efficiencies of the first coverglass preparations were 8 and 53 in sodium chloride and 52 and 90 in zinc sulphate solution.

The specific gravity of eggs of the dog whipworm (*Trichuris vulpis*) was tested in 2 experiments. The amounts of suspension used were 0.3 and 0.5 ml containing approximately 80 and 100 eggs respectively. The summarized results are presented in Table 3.

TABLE 3.—Percentages of recovery of *Trichuris vulpis* eggs from different levels of $ZnSO_4$ solutions of various specific gravities. Summary of 2 experiments

Specific gravity	1.140	1.150	1.160	1.180	1.200
Surface layer	0	37	88	92	100
Supernatant liquid ..	0	0	0	0	0
Sediment	100	63	12	8	0

Eggs of the dog hookworm (*Ancylostoma caninum*).—The specific gravity of eggs of *Ancylostoma caninum* was tested in 2 experiments. The amounts of suspension used were 0.5 and 0.4 ml containing approximately 200 and 180 eggs respectively. The summarized results are presented in Table 4.

TABLE 4.—Percentages of recovery of *Ancylostoma caninum* eggs from different levels of $ZnSO_4$ solutions of various specific gravities. Summary of 2 experiments

Specific gravity	1.040	1.055	1.150	1.180	1.200
Surface layer	0	38	92	100	99
Supernatant liquid ..	1	10	0	0	0
Sediment	99	52	8	0	1

For these *Ancylostoma caninum* eggs the efficiency percentages of the first coverglass preparations removed from the surface layers of the solutions with the various specific gravities were as follows: specific gravity 1.040, 0; 1.055, 21; 1.150, 81; 1.180, 87; and 1.200, 92.

Eggs of *Ascaris lumbricoides*.—The specific gravity of fertilized *Ascaris lumbricoides* eggs was tested in 5 experiments. The amounts of suspension used were 0.1, 0.1, 0.1, 0.2, 0.4 ml containing approximately 250, 300, 500, 600 and 450 eggs. The results in these experiments differed considerably; the range in results is presented in Table 5, the

TABLE 5.—Percentages of recovery of fertilized *Ascaris lumbricoides* eggs from different levels of $ZnSO_4$ solutions of various specific gravities. Range of results in 5 experiments

Specific gravity	1.080	1.100	1.110	1.120	1.140	1.180	1.200
Surface layer	0	0-1	1-56	25-97	90-99	97-100	98-100
Supernatant { upper ..	0	0	0-6	5-0	2-0	0	0
{ lower ..	0	0	0-4	4-0	0	0	0
Sediment	100	100-99	99-34	66-3	8-1	3-0	2-0

lower percentages being obtained with 500 eggs in each test tube, the higher ones with 300 eggs.

For these fertilized *Ascaris* eggs the efficiency percentages of the first coverglass preparations removed from the surface layers of the solutions with the various specific gravities ranged as follows: specific gravities 1.080 and 1.100, 0; 1.110, 1 to 32; 1.120, 6 to 73; 1.140, 53 to 71; 1.180, 57 to 79 and 1.200, 94. A top speed centrifugation time of 50 and 280 seconds did not essentially alter the results.

The specific gravity of unfertilized eggs of *Ascaris lumbricoides* was tested in 2 experiments. The amounts of suspension used were 0.2 and 0.4 ml containing approximately 90 and 120 eggs. The summarized results are presented in Table 6.

TABLE 6.—Percentages of recovery of unfertilized *Ascaris lumbricoides* eggs from different levels of $ZnSO_4$ solutions of various specific gravities. Summary of 2 experiments

Specific gravity	1.160	1.180	1.200	1.250
Surface layer	0	3	39	99
Supernatant liquid	0	2	13	0
Sediment	100	95	48	1

DISCUSSION

The specific gravities of eggs of the pinworm, the whipworm, the hookworm and *Ascaris* were tested in zinc sulphate solutions of various specific gravities by centrifugal floatation. Zinc sulphate and centrifugal floatation were chosen in order to obtain additional quantitative evaluation of the zinc sulphate centrifugal floatation technic (3, 4). Buoyancy tests are used for the determination of specific gravity values. However, the difference in yield of whipworm eggs from sodium chloride and zinc sulphate solutions of the same specific gravity adds evidence that the specific gravity of a biologic object determined by its buoyancy is not necessarily identical with its true specific gravity, due to the possible interaction of floating medium and object (2). The zinc sulphate medium apparently interferes less, possibly by virtue of the difficulty of the sulphate ion to penetrate the living cell membrane (5).

For the floatation technic a solution of a specific gravity equal to that of the eggs is not sufficient. The specific gravity of the floating medium must be higher. The difference in the two values influences the number of eggs buoyed up and especially the number recovered from the first coverglass preparation. A centrifugation time of 100 seconds top speed was employed after it was found that 50 and 280 seconds provided lower yields on the first coverglass preparation. However, it is important to note that the manner in which the superimposed coverglass is lifted from the tubes influences the yield, as emphasized by Lane (6).

While few if any eggs of *Enterobius vermicularis* floated in solutions of specific gravities of 1.110 and lower, in a solution of 1.115 the eggs were distributed in the surface layer, the supernatant liquid and the sedi-

ment. In solutions of higher specific gravity the top layer yield increased. Thus, the specific gravity of *Enterobius vermicularis* eggs, as determined by buoyancy tests in zinc sulphate solutions, is approximately 1.115. For floatation technics a solution of this specific gravity obviously does not provide a sufficient yield. Only 57 per cent of the total number of eggs was found in the surface layer, and only 12 per cent on the first coverglass preparation. While a solution of the specific gravity 1.180 will float almost all of the *Enterobius vermicularis* eggs, the first coverglass preparation yielded little more than two-thirds of the total number.

In solutions of specific gravity 1.140 and lower only an occasional egg of *Trichuris trichiura* or *Trichuris vulpis* floated; in solutions with specific gravities 1.150 and 1.160 the eggs were distributed in the surface layer, the supernatant liquid and the sediment. Thus, the specific gravity of *T. trichiura* eggs, as determined by buoyancy tests, appears to be between 1.150 and 1.160.

Solutions of higher specific gravity have to be used for concentration of these eggs. In a solution of specific gravity 1.180, 85 per cent of the total number was recovered from the surface layer by consecutive coverglass preparations, although the first preparation yielded only one half of the total number present. A first coverglass yield of 90 per cent was obtained with a solution of specific gravity 1.200. These yields are in accordance with results obtained by Faust et al. in a qualitative study (4), in which more than 10 per cent of infections actually present were missed, when centrifugal floatation in zinc sulphate solution of specific gravity 1.180 was employed. The specific gravity values of *T. vulpis* eggs were similar to those of *T. trichiura*.

Lane (6) found a sodium chloride solution of specific gravity 1.150 inadequate for floating whipworm eggs that had been preserved under water for more than 4 weeks. A sodium chloride solution of specific gravity 1.200 floated approximately one-half the number that floated in calcium chloride solutions of specific gravity 1.300 and higher. These findings indicate that old whipworm eggs in these salt solutions need a greater buoyant force than do fresh eggs in zinc sulphate solutions.

Eggs of *Ancylostoma caninum* were found distributed in the surface layer, the supernatant liquid and the sediment in zinc sulphate solutions of specific gravity 1.055, thus suggesting that this is their specific gravity. This value agrees exactly with that of *Necator americanus* eggs previously reported (2). While in solutions of specific gravity 1.150 more than 90 per cent of *A. caninum* eggs was recovered from consecutive preparations removed from the surface layer, the efficiency of the first coverglass preparation was 80 per cent. Heavier solutions were still more efficient, solutions of specific gravity 1.180 yielding 87 per cent.

Lane (7) has stressed the need of using a solution having at least a

specific gravity of 1.150 for the practical purpose of concentrating human hookworm eggs by floatation. The data presented for *A. caninum* are in agreement with Lane's findings. They are further substantiated by the following unpublished results obtained during the study of the specific gravity of *Necator americanus* eggs (2): The efficiency of the first coverglass preparations from the surface layers of solutions of specific gravities 1.045 and 1.050 was 0; of 1.055, 4 per cent; of 1.060, 53 per cent; of 1.065, 56 per cent and 1.180, 98 per cent.

Fertilized eggs of *Ascaris lumbricoides* in the one-cell stage varied in their specific gravity values. In one series of experiments the eggs were buoyed up in solutions of specific gravity 1.120 to 1.130 and in another, of 1.110, so that 1.110 to 1.130 may be considered the range. On the first coverglass preparation, when zinc sulphate solution of specific gravity 1.180 was used, only 57 to 79 per cent of the total yield was recovered, while a solution of specific gravity 1.200 yielded 94 per cent. Unfertilized eggs of *Ascaris* floated in solutions of specific gravity 1.200, but more readily in solutions of specific gravity 1.250.

Lane (6) found that only 4 per cent of fertilized *Ascaris* eggs floated in a sodium chloride solution of specific gravity 1.150, while in a solution of specific gravity 1.200, about one half the number floated as with a calcium chloride solution of specific gravity 1.300. However, these results were obtained with eggs more than 4 weeks after being passed and in solutions other than zinc sulphate. In 1926, Sasaki (1) reported the specific gravity of fertilized *Ascaris* eggs as varying between 1.083 and 1.187, the less mature eggs being heavier than the more mature ones, unfertilized eggs being still lighter. The difference between Sasaki's findings and those reported here might be due to different floating media employed. The information necessary for comparison was not available, since the report is written in Japanese.

SUMMARY

The specific gravities of several nematode eggs have been tested in zinc sulphate solutions of various specific gravities. However, values thus determined are not necessarily the actual specific gravities. For the concentration of these eggs by centrifugal floatation a solution of a specific gravity higher than their own is necessary. The centrifugation time is a function, mathematically expressed, of the difference between the specific gravities of the eggs and the solution.

Enterobius vermicularis eggs were buoyed up in solutions of specific gravity 1.115 and higher. A solution of specific gravity 1.180 yielded 97 per cent of the total in the surface layer and 70 per cent on the first coverglass preparation.

Trichuris trichiura eggs were buoyed up in solutions of specific grav-

ity 1.150 and higher. A solution of specific gravity 1.180 yielded 85 per cent of the total in the surface layer and 52 per cent on the first coverglass preparation, while a solution of specific gravity 1.200 yielded 99 and 90 per cent respectively.

Trichuris vulpis eggs were buoyed up in solutions of specific gravity 1.150 and higher.

Ancylostoma caninum eggs were buoyed up in solutions of specific gravity 1.055 and higher. A solution of specific gravity 1.150 floated 92 per cent of the eggs and a solution of specific gravity 1.180, 100 per cent, with an efficiency of the first coverglass preparation of 87 per cent.

Fertilized *Ascaris lumbricoides* eggs differed in their buoyancy in different experiments within the range 1.110 to 1.130. A solution of specific gravity 1.180 floated almost all of these eggs, while the efficiency of the first coverglass preparation varied between 57 and 79 per cent. A solution of specific gravity 1.200 yielded 94 per cent on the first coverglass preparation.

Only 39 per cent of unfertilized *Ascaris lumbricoides* eggs was buoyed up in solutions of specific gravity 1.200, while a specific gravity of 1.250 yielded 99 per cent.

The data on the specific gravity of these eggs provide basic information on the required specific gravity of the solution to be employed for floatation technics. The differences in the specific gravities of the eggs examined suggest the possibility of differential floatation.

BIBLIOGRAPHY

1. SASAKI, R. 1927 On the oscillation of specific gravity of the *Ascaris* eggs which are manifested by their growth. Tokyo Iji-Shinshi (Japanese text). English summary in Japan Med. World 7: 115.
2. SAWITZ, W., TOBIE, J. E. AND KATZ, G. 1939 The specific gravity of hookworm eggs. Am. J. Trop. Med. 19: 171-179.
3. FAUST, E. C., D'ANTONI, J. S., ODOM, V., MILLER, M. J., PERES, C., SAWITZ, W., THOMEN, L. F., TOBIE, J. E. AND WALKER, J. H. 1938 A critical study of clinical laboratory technics for the diagnosis of protozoan cysts and helminth eggs in feces. Am. J. Trop. Med. 18: 169-183.
4. FAUST, E. C., SAWITZ, W., TOBIE, J. E., ODOM, V., PERES, C. AND LINCICOME, D. R. 1939 Comparative efficiency of various technics for the diagnosis of protozoa and helminths in feces. J. Parasitol. 25: 241-262.
5. KROGH, A. 1939 Osmotic regulation in aquatic animals. Cambridge University Press. p. 35.
6. LANE, C. 1925 The mass diagnosis of ankylostome infestation. Part XIV. Criticisms, constructive and otherwise. Tr. Roy. Soc. Trop. Med. and Hyg. 19: 156-176.
7. ——— 1940 Hookworm diagnosis. Assumptions, alterations, controls, time-lag, rediscoveries: D.C.F. Tr. Roy. Soc. Trop. Med. and Hyg. 33: 521-536.

STUDIES ON DERMATITIS-PRODUCING SCHISTOSOMES
IN EASTERN MASSACHUSETTS, WITH EMPHASIS
ON THE STATUS OF *SCHISTOSOMATIUM*
PATHLOCOPTICUM TANABE, 1923¹

LAWRENCE R. PENNER²

Department of Comparative Pathology and Tropical Medicine,
Schools of Medicine and Public Health, Harvard University

During the summer of 1940 it was possible to study the potential or real schistosome dermatitis problems which occur in eastern Massachusetts. The center of operations was the Harvard Medical School at Boston, where Tanabe, early worker on rodent schistosomes in the United States, had studied. Not only was the prevalence of the causative organisms in the region investigated but five of Tanabe's original slides of *Schistosomatum pathlopticum* were studied.

HISTORICAL

Tanabe (1923) infected white mice and white rats with a schistosome cercaria found in *Stagnicola palustris* collected in the vicinity of Boston, Massachusetts, primarily from the Back Bay Fens region. Because the adult worms were different from any previously described, he erected a new genus and species, *Schistosomatum pathlopticum*, for his specimens. This was the first schistosome life cycle to be completed experimentally in this country. He was unable however, to demonstrate a natural host for the worm.

Price (1929) described a new adult schistosome obtained experimentally in laboratory rats and mice. She produced the infection by experimental exposures to *Cercaria douthitti* Cort, 1914, from the snails, *Lymnaea stagnalis apressa* Say and *S. palustris* (Müller). Later Price (1931) reported quite fully the entire life cycle and believed field mice to be the natural hosts of the fluke. She considered the worm to be very closely related to *S. pathlopticum* but because of certain differences she discovered in her study, did not believe them synonyms. Price was able to infect *Peromyscus maniculatus* (Wagner) Bangs, *Mus musculus* Linnaeus, *Microtus pennsylvanicus* (Ord.), white mice, white

Received for publication, April 22, 1941.

¹ Aided by a grant from the American Academy of Arts and Sciences.

² Appreciation is expressed to Dr. Donald L. Augustine for allowing this work to be conducted in his research laboratory; to Dr. E. E. Tyzzer for his kindly suggestions and help; to Dr. A. Watson Sellards for loaning me slides of *Schistosomatum pathlopticum* Tanabe, which had been given to him in 1923 by Dr. Tanabe; and to Dr. Joseph C. Bequaert and Mr. W. J. Clench for determining the species of snails collected during the survey.

rats, and a cat. Her examinations of several species of mammals from nature were negative with the exception of two meadow mice, one of which had one pair of worms in a mesenteric vein of the cecum while the other had three males and one female in a similar position. The animals were collected in the region of Third Sister Lake, near Ann Arbor, Michigan.

The above were the only records of natural infections until Penner (1938) tentatively determined as *Schistosomatium douthitti* (Cort) worms found in 32 out of 330 muskrats from the vicinity of Minneapolis, Minnesota, examined during March and April, 1938, and in one of two muskrats examined at the University of Michigan Biological Station during August, 1938. Since that time Penner (1939, 1940) has experimentally infected muskrat, guinea pig, snow-shoe hare, and cottontail and laboratory rabbits with *C. douthitti* and has also collected data on the role played by the muskrat as the natural definitive host. Since the spring of 1938, 374 muskrats in the vicinity of Minneapolis, have been examined for schistosomes. Forty-two (11.2%) were found infected. One of two brown rats examined November 6, 1939, had in the liver spineless schistosome eggs which were identified as those of *S. douthitti*. The rat was caught in water about two feet from shore in a baited live trap. Most of the eggs were walled off in the liver with fibrous tissue and no eggs were found in the feces of the rat. Mr. A. C. Cuckler, in a recent personal communication, reported finding schistosomes in *Microtus* trapped during July, 1940, at a pond near Edina, Minnesota. Although they have not been studied, the worms are likely *S. douthitti*, as extensive snail collecting in the pond has failed to reveal any species of schistosome cercariae other than *C. douthitti*.

Porter (1938) reported *Schistosomatium pathlocopticum* in experimental mice at Johannesburg, South Africa. She exposed a month old mouse to cercariae from *Bulinus tropicus* for 45 minutes and at the death of the mouse in 32 days, found one male and one female worm which agreed in many particulars with the adults found by Tanabe (1923). However, her measurements, especially of suckers, are somewhat at variance with measurements of either *S. pathlocopticum* as described by Tanabe, or *S. douthitti*. The material with which she worked was limited and it is possible that she was dealing with an entirely different species of rodent schistosome.

McLeod (1940) recorded the examination of 460 snow-shoe hares (*Lepus americanus*), 240 gophers (*Citellus*, 3 spp.), and 36 muskrats in the laboratories of the University of Manitoba but all were negative for schistosomes. Other examinations of rodents in this country have failed to reveal schistosomes.

THE STATUS OF *Schistosomatum pathlocopticum* TANABE, 1923

One of the main purposes of the study of dermatitis-producing schistosome cercariae in the vicinity of Boston was to determine the relationship of Tanabe's species, *S. pathlocopticum*, to *S. douthitti*. Extensive collecting failed to reveal any schistosome cercariae which could be considered identical with the cercariae for either species as described. Therefore, before going further with the problem of schistosome dermatitis in Massachusetts it is deemed advisable to discuss the status of *S. pathlocopticum* as determined from Tanabe's publications and from some of his preserved material consisting of five slides labelled as follows: C. B. Cercaria; Male from mesenteric v. of mouse 33 days after infection July 7, 1922; ♀ from mes. vein of ms. 25 days after infection July 15, '22; Intestine of Ms. B; and Eggs from Liver of Ms. G. July 21, 1922. From a thorough study of the slides and of the results from certain experiments I have come to the conclusion that Tanabe's species is a synonym of *S. douthitti* (Cort, 1914). The evidence to warrant the above conclusion is incorporated in the following discussion.

ADULT WORMS

Tanabe reported the maximum length of the males of *S. pathlocopticum* to be 11.8 mm and that of the females, 10.2 mm. The age of the infection was not given. These measurements are considerably longer than those given by other authors for specimens of *Schistosomatum*. Penner (1940) found the maximum length of males of *S. douthitti* in white mice to be 7.14 mm, and that of the females, 4.53 mm. This in itself, however, is not sufficient reason for separating it from *S. douthitti* as there may be several other causes of length difference, as for example:

a. Distortion of the specimens on extraction from host. I have been able to extend specimens of *S. douthitti* to more than 10 mm when extracting them from the mesenteric vessels of mice dead several hours before autopsy. It is suggested that Tanabe's longest worms may have been removed when dead.

b. Intensity of infection affects the size of the worm (Price, 1931). In my experiments the largest worms have generally been found in lightly infected mice.

c. That there may be remarkable differences in the size of worms has been recognized for other species of schistosomes. Cort (1923) found quite a range of variation in specimens of *S. japonicum* recovered on any given day after infection. In general, the same has been found true of *S. douthitti* (Penner, 1940).

d. The length of time the infection has been allowed to run before the host is autopsied. Cort (1921) in studying *S. japonicum* noted remark-

able changes in size as the worms passed from the cercarial to the adult stage, the worms increasing as much as 130 times their cercarial size before becoming mature adults. Specimens of *S. japonicum* from mice, although usually smaller, have reached the size of 8 mm for males and 11 mm for females from hosts examined 62 days after exposure (Katsurada, 1914).

e. In addition, the age of the host, the strain of laboratory animal used, individual resistance or susceptibility of the host to infection, and diet of the host are among the factors which may influence the size of the worm.

By experimentally infecting mice with cercariae of *S. douthitti* and performing autopsies at lengths of time corresponding to those of Tanabe's infections the author was able to obtain comparable measurements (Table 1).

TABLE 1.—Comparative maximum measurements, in millimeters, of male *Schistosomium* taken from experimental infections of white mice

Days	Tanabe (1923) Cercariae from <i>S. palustris</i>	Penner (present paper)
12	1.658	1.20 (cercariae from <i>L. stagnalis</i>)
21	3.85	3.84 (cercariae from <i>S. palustris</i>)
24	5.785	
27		5.04 (cercariae from <i>L. stagnalis</i>)
30	6.338	7.14 (cercariae from <i>L. stagnalis</i>)
	Maximum measurements of females	
21	3.851	3.18 (cercariae from <i>S. palustris</i>)

In my experimental infections the mice were exposed by being placed in a bell jar containing water with cercariae. The time of exposure varied from 4–20 minutes depending on the number of cercariae used. The water was deep enough so the feet and lower parts of the mice were under water.

In Tanabe's mounted material which I have had the opportunity of studying, there are two slides of adult worms, one containing two males from a 33 day infection in mice, and the other containing three females from a 25 day infection in mice. The specimens were apparently mounted in balsam and ringed with some kind of sealing cement. The mounting medium has partially evaporated thereby distorting the specimens to a certain degree. They are, however, still satisfactory for use in observing general morphological features.

In order to have *S. douthitti* specimens of the same age, mice were infected with known *S. douthitti* cercariae and autopsied at dates corresponding to those on Tanabe's slides. Measurements are indicated in Table 2.

The measurements of Tanabe's specimens are for the most part in agreement with those given for *S. douthitti*. Such differences as do exist, can be accounted for by the excessive flattening caused by the drying of his slides over a period of several years.

TABLE 2.—Measurements, in millimeters, of Tanabe's mounted specimens as compared with those of known *S. douthitti* adults of the same ages

♂♂	33 days Tanabe (2 specimens)	33 days Penner (10 specimens) *
Body length	5.7-7.14	3.4-7.12
Length forebody	2.7-3.14	1.4-2.4
Width forebody	0.43-0.56	0.36-0.60
Length hindbody	3.97-3.99	1.74-4.72
Width hindbody	0.61-0.65	0.21-0.54
Length oral sucker	0.16-0.16	0.12-0.18
Length ventral sucker	0.14-0.22	0.18-0.20
Oral s. to ventral s.	0.96-1.11	0.57-0.84
♀♀	25 days Tanabe (3 specimens)	25 days Penner (10 specimens) *
Body length	4.5-6.3	1.92-4.50
Body width	0.18-0.31	0.15-0.30
Oral s. length	0.09-0.14	0.09-0.11
Ventral s. length	0.14-0.18	0.09-0.13
Oral s. to ventral s.	0.41-0.45	0.30-0.42

* Snail host, *Stagnicola reflexa*.

Another character which has been used to separate *S. pathlocopticum* from *S. douthitti* has been testicular lobation. Tanabe's reports for *S. pathlocopticum* were 14-16 follicles and Price (1931) for *S. douthitti* 15-36. However, the two males in Tanabe's material had 20 and 22 distinct follicles with several smaller lobules which are not so distinct. The testes of a 33 day old male *S. douthitti* from an experimental infection in white mouse are quite similar. Tanabe apparently erred in counting the testicular lobation in his own experimental material. I have found all gradations of testicular lobation in experimental material from a lobed mass to as many as 29 follicles. The situation seems to depend largely on the duration of the infection. For example, as few as 15 lobes have been counted in a 21 day infection and as many as 29 in a 130 day infection in laboratory mice.

It is interesting to note in this respect that Porter (1938) in reporting *S. pathlocopticum* from South Africa, figured and indicated that the male worm she studied, had 14 distinct testicular lobules. If the lobation is found constant after more material from South Africa is studied, it may prove significant in determining the status of Porter's specimens. It is possible, as mentioned before in this paper, that, provided the adult and cercarial morphology are as she described them, she was dealing with a new species.

EGG

The section of liver in Tanabe's material marked "Eggs from Liver of Ms. G, July 21, 1922," is not very satisfactory for measuring the eggs. A series of 10 ranged from 45-62 microns in length and 42-50 microns in width. Table 3 gives comparative measurements of *Schistosomatum* eggs as recorded by different authors. It also indicates the variation in size of eggs as they are found in different habitats. The

TABLE 3.—Comparative measurements, in microns, of *Schistosomium* eggs as recorded by different authors

Location of eggs	Tanabe, 1923 10 eggs (mouse)	Price, 1931 50 eggs (mouse)	Penner, present paper 50 eggs (host- muskrat)
Uterus of female worm...	40- 59 long	42- 80 × 30-58	35- 90 × 30-65
Liver of definitive host...	56- 98 × 43-70		50-106 × 40-76
Intestinal wall	49- 75 × 56-87		44-110 × 54-90
Mature or developed egg	80-126 × 58-87	94-122 × 74-98	90-128 × 64-96

developed eggs were found by Price (1931) to occur 28-30 days after the definitive host was exposed to infection. The measurements do not indicate that the material studied might be of more than one species of fluke.

CERCARIA

Tanabe (1923) indicated the close resemblance of the cercaria of *S. pathlocopticum* to *C. douthitti*. The major difference was in the recorded number of penetration glands. Tanabe reported three pairs present in *S. pathlocopticum*, this number being verified, perhaps erroneously, by Porter (1938). Cort (1914) first described *C. douthitti* as having 4 pairs of penetration glands, but Cort (1917) and others have since observed 5 pairs, while Price (1931) added an extra pair of escape glands present in the cercaria before emergence from the daughter sporocyst. Miller (1936) in his key to the Illinois cercariae still retained Cort's (1914) first observation that four penetration glands are present on each side of the body in *C. douthitti*.

Tanabe's slide labelled "C.B. Cercaria" contains 7 cercariae, five of which are considerably flattened and more or less separated from the mounting fluid while the other two are but slightly distorted and in the mounting medium. Several of the cercariae show what appear to be 5 pairs of glands with ducts leading away from them.

After examining this material, I am convinced that Tanabe's material has 5 pairs of penetration glands and feel that he erred in determining the actual number present. Porter (1938) perhaps erred in her study of cercariae which she determined to be *S. pathlocopticum*. Although she reported only 3 pairs of penetration glands, it is quite possible that she did not have enough available material to correctly determine the actual number of glands. Her drawings resemble those published by Tanabe (1923). If there are only 3 pairs of penetration glands in her material, she is dealing with a new species of schistosome worm.

There are 5 or 6 penetration spines on each side of the concavity of the head organ near the openings of the ducts from the penetration glands. Tanabe (1923) described distinct anterior spines on his cercaria, believing their presence signified a difference between the cercariae

of *S. pathlocopticum* and *S. douthitti*. As *C. douthitti* has such spines, this was not a valid difference.

C. douthitti was first described without cuticular spines (Cort, 1914). Later Cort (1917) restudied the species and observed their presence under high power of the microscope. Tanabe (1923) recorded minute spines covering the whole surface of the tail and body in the material he studied and other workers have demonstrated the same for *C. douthitti*.

The flame cell pattern as given by Cort (1917) and Price (1931) is the same as that given by Tanabe (1923). There are 5 pairs of body flame cells and 1 pair in the tail. Porter (1938) erroneously cited the

TABLE 4.—Comparative measurements, in millimeters, of *Schistosomium cercariae* as given by different authors

	Cort, 1914, 1917, mounted	Tanabe, 1923, 10 speci- mens, 5% formalin	Penner, present paper, 7 of Tanabe's mounted specimens	Price, 1931, 25 speci- mens, 10% formalin	Porter, 1938, 10 speci- mens, living	Penner, present paper, 20 specimens, 10% for- malin. Cercariae from <i>L. stagnalis</i>	Penner, present paper, 20 specimens, 10% for- malin. Cercariae from <i>Stagnicola refusa</i> (Say)
Av. body length	.190	.18	.194 (.15-.23)	.204 (.186-.24)	(.115-.18)	.200 (.188-.240)	.195 (.186-.228)
Av. body width	.067	.08	.091 (.06-.10)	.068 (.057-.081)	(.068-.082)	.072	.074
Av. tail stem length	.220	.23	.170 (.14-.18)	.210 (.186-.234)	(.133-.220)	.215	.221
Av. tail stem width	.025	(basal) .045	.035 (.17-.46)	.025	(.036-.043)	.025	.025
Furcal rami length	.089	.10	.064 (.038-.154)	.096 (.08-.11)	(.06-.103)	.092	.095
Head organ length	.057	.05	.059 (.38-.74)	.076 (.067-.082)	(.042-.045)	.071	.070
Head organ width	.045	.047	.062 (.38-.77)	.048 (.038-.062)		.046	.047
Acetabulum diameter	.025	.024	.028 (.02-.035)	.025 (.019-.028)	.03	.025	.025
Eyespots	.007	.008	.008 (.007-.009)	.007 (.006-.009)		.007	.007 (.006-.009)
Pairs penetration glands	4 (1914) 5 (1917)	3	5	5 (6)	3	5	5 (6)
Pairs flame cells	6 (1917)	6	6	6	6	6	6
Anterior spines	Not desc. (1914) Desc. (1917)	Present	Present	Present	Present	Present	Present

cercariae of *S. douthitti* as having 10 flame cells on each side of the body rather than 5, and used this as a difference between *S. pathlocopticum* and *S. douthitti*.

In general Tanabe's observations agree with Price's very complete description of the cercaria of *S. douthitti*. Measurements and other fundamental data are such as to indicate that Tanabe's cercaria is *C. douthitti*. Size, eyespots, spination, etc. are all in agreement with *C. douthitti* of other authors. In order to indicate the essentials without elaborate discussion Table 4 incorporates comparative measurements of *Schistosomatium* cercariae as given by different authors and my measurements of Tanabe's slide material.

From Table 4 it can be discerned that the measurements of Tanabe's mounted specimens and Tanabe's own personal measurements are such as to conform to those given for the cercariae of *S. douthitti*. In Table 5

TABLE 5.—Measurements, in microns, of Tanabe's permanent cercarial mounts

	1	2	3	4	5	6	7
Body length	193	192	228	217	224	154	151
Body width	105	98	105	102	105	63	60
Length head organ....	60	59	74	70	73	39	38
Width head organ....	68	70	70	70	77	42	38
Tail stem length.....	172	140	175	154	178	182	178
Tail stem width.....	35	39	38	42	46	17	25
Furcal rami length....	52 &	38 &	59 &	67 &	91 &	49 &	46
	56	42	68	70	154	53	
Furcal rami width	14	14	21	21	21	7	7
Ventral sucker length..	14	17	28	28	28	21	21
Ventral sucker width..	21	21	32	35	35	28	28
Eyespots	8	9	8	8	8	8	7
Penetration glands	5 pr?	5 pr?	5 pr?	5 pr?	5 pr	?	?
Penetration gland ducts	5?	5?	?	?	?	5?	?

the measurements in microns were used in determining the averages of the mounted cercariae on Tanabe's permanent mount of the cercariae. The last two columns are measurements of the specimens not excessively flattened and still in the mounting fluid on the slide. The first five columns include the somewhat flattened specimens.

The only other cercaria which may be placed in very close proximity with the *Schistosomatium* material of North America and South Africa is *Cercaria "C"* Kemp, 1921, knowledge of which is confined to data from preserved material. The latter material from India was placed in Miller's (1924) apharyngeal brevifurcate distome cercariae Group C (*douthitti* of Sewell, in part), along with *C. douthitti* and the cercaria of *S. pathlocopticum*.

Emergence of cercariae from the snail host. Tanabe (1923) observed great numbers of the cercariae of *S. pathlocopticum* discharged daily from infected snails into the surrounding water "—especially on cloudy days and nights, or in clear, recently renewed water." My observations and the observations of other authors on *C. douthitti* fit the observations of Tanabe in these respects. For example Penner (1940)

found that on one very dark day, cercariae of *S. douthitti* from one *Stagnicola reflexa* (Say) emerged at 3:30 PM, whereas on other fair days, emergence occurred from 5:30 to 9:30 PM. Cort and Talbot (1936) found that cercariae of *S. douthitti* began to escape from their snail hosts from 8:30 to 9:30 PM and that almost all that would escape during a 24 hour period would be out within an hour.

Behavior of the cercariae. According to Tanabe, not nearly such a large proportion of the cercariae of *S. pathlocopticum* as of *S. douthitti* remain attached to the surface film during their free life. *C. douthitti* has been found by many authors to spend most of its free life attached to the surface film of the water; nevertheless, on studying emergence, I have found *C. douthitti* free in the water, especially if the bottle is handled or jarred, although invariably the cercariae are later attracted to the surface film. It is quite a common occurrence to note the cercariae free in the water, especially after a change of water on the infected snail. These observations on *C. douthitti* are quite in agreement with Tanabe's observations on what he believed to be a new species.

RESULTS OF INVESTIGATIONS ON THE PRESENCE OF DERMATITIS-PRODUCING SCHISTOSOME CERCARIAE IN EASTERN MASSACHUSETTS
DURING THE SUMMER OF 1940

As the cercariae of *S. douthitti* are known to produce schistosome dermatitis or water itch, and Tanabe's species is really *S. douthitti*, eastern Massachusetts already has one schistosome cercaria which can produce water itch. Although other schistosome cercariae have been reported from Massachusetts (Rankin, 1939) none is known to be a dermatitis producer.

Since the main purpose of my study of dermatitis-producing schistosomes in eastern Massachusetts was to straighten out Tanabe's material, it was considered advisable to collect fresh-water snails in areas reported by Tanabe to contain schistosome infected snails. Although Tanabe indicated that his collections were made solely in the Back Bay Fens of Boston, Mr. W. J. Clench, Curator of the Museum of Comparative Zoology at Harvard University, informed me that Tanabe made collections in different parts of eastern Massachusetts other than in the Back Bay Fens region. Because of this, my collections were extended to include accessible waterways that Tanabe might have reached, as well as to include lakes and other areas where potential or real dermatitis problems might prevail. Likewise, collections were made in areas where *Stagnicola palustris* (Müller) had been reported. This information was obtained from the distribution data of the Peabody Museum of Comparative Zoology Mollusc Collection at Harvard University. Information on the distribution of other snails which might be hosts of schistosome cercariae, was also obtained from these records.

Collections were made in various areas arbitrarily designated as follows: Muddy River and Charles River Basin Area (Jamaica Pond through Ward's Pond, Leverett Pond to the Charles River), Hammond's Pond Area (Hammond's Pond, Chestnut Hill Reservoir, Lost Pond), Dedham Area (Charles River two miles on either side of Dedham, Motley Pond, Wigwam Pond, and Mother Brook), Newton Area (Newton, Newton Center, Newtonville, Charles River near Newton Upper Falls and Newton Lower Falls, and West Newton), Cambridge Area (Fresh Pond, Perch Pond, Little River, portion of Alewife Brook and several clay pits), Arlington and West Medford Area (Little Spy Pond, Spy Pond, lower portion of Alewife Brook, Upper and Lower Mystic Lakes), Wakefield Area (Crystal Lake, Lake Quannapowitt, a portion of Saugus and Mill Rivers, and Dark Meadows), Wellesley Area (Lake Waban, Morse's Pond, Jennings's Pond, and None Such Pond), Auburndale Area (Weston and Cambridge Reservoirs, Charles River from Auburndale to Waltham), and Cape Cod Area (Great Herring, Pickerel, Mashpee, Wakeby, Santuit, Lovell's, Long, Shiverick, Sider's, Falmouth Dump, Sandwich Mill, Sandwich Fish Hatchery, and several other smaller unnamed ponds).

Despite extensive collecting during July and August in the Back Bay Fens where Tanabe reported finding infected *S. palustris*, I was unable to collect this species of snail in that immediate region. That the snail or its eggs might be washed into the region is possible because 28 young and adult *S. palustris* were collected at the anterior end of Leverett Pond and 1243 specimens were also taken in a small pool between Leverett and Ward's Pond. After 1 to 3 days isolation in 8 ounce bottles with no schistosome cercariae appearing, the snails were crushed to determine whether larval trematode infections were present. All snails were negative for schistosome cercariae. One hundred and forty of the area's most commonly observed snail, i.e., *Viviparus malleatus* (Reeve)—large imported operculate Japanese snail—were examined for trematode larvae but found negative. One *Physella heterostrophia* (Say) out of 1757 collected and examined, was found to have an immature schistosome infection. It was not however of the *Schistosomatium* type but was similar to *Cercaria physellae* Talbot, 1936, and other closely related species known to be dermatitis producers. Collections of 215 *Gyraulus parvus* (Say), 200 *Amnicola limosa* (Say), 139 *Helisoma trivolvis* (Say), 123 *Galba humilis modicella* (Say), 108 *G. umbilicata* C. B. Adams, 65 *Radix auricularia* (Linnaeus)—an imported European snail and 14 *Pseudosuccinea columella* (Say) were made from various areas along the Muddy River and upon resultant examination found negative for schistosome infections.

Almost no snails were found in Hammond's Pond Area with the

exception of Hammond's Pond. Collections of 145 *Physella heterostropha*, 8 *Pseudosuccinea columella*, 9 *Planorbula jenkinsii* Carpenter, and 120 *Gyraulus parvus* were made and on later examination were found negative for schistosomes.

The Dedham Area was found almost barren of snails with the exception of several *Pseudosuccinea columella* in Wigwam Pond. These upon examination were found negative for schistosome infection.

Collections in all but one region of the Newton Area failed to reveal snails in significant number. An examination of 1000 *Stagnicola palustris* and 2000 *Physella heterostropha* from a small pond and stream near the crossing of Commonwealth and Walnut Avenues failed to reveal any schistosomes. Likewise, 124 *Physella heterostropha* and 65 *Helisoma trivolvis* collected below Bullough's Skating Rink Pond were negative for schistosomes.

In the Cambridge Area many *Viviparus malleatus* and *V. contectoides* Binney—operculate imported Japanese snails—*Physella heterostropha* and a few *Helisoma trivolvis* were collected from a pond on a golf course near Fresh Pond; 500 *Stagnicola palustris* from a small pool located near the Hills Crossing Station; and a few *Physella heterostropha*, *Viviparus malleatus*, and *Radix auricularia* from Perch Pond in the vicinity of Little River. All of these snails were found negative for schistosome cercariae.

In the Arlington and West Medford Area 500 *Physella heterostropha* were collected from Little Spy Pond, 250 *Galba humilis modicella* from a small brook draining into Little Spy Pond, 42 *Physella heterostropha* from Spy Pond, and 3 *Stagnicola palustris* from an outlet to a storm sewer on the east side of Lower Mystic Lake. All snails upon examination were found negative for schistosome infections.

In no region of the Wakefield Area is an abundant snail population found. Twenty-six *Physella heterostropha* were collected in Crystal Lake; a few *P. heterostropha* and *Helisoma trivolvis* from the Colonial Golf Club, 65 *Physella heterostropha* and 23 *Pseudosuccinea columella* from Saugus and Mill Rivers. On examination all were found negative for schistosome cercariae.

Snails were not abundant in any of the regions of Wellesley Area. No schistosome cercariae were found in 126 *Gyraulus parvus* and 38 *Physella heterostropha* collected on the Wellesley College side of Lake Waban nor in 46 *Gyraulus deflectus* (Say) collected in Jennings's Pond.

Snails were not found in sufficient numbers for collection in the Auburndale Area.

In the Cape Cod Area no schistosome cercariae were found in an examination of 251 *Gyraulus deflectus* (Say), 36 *Amnicola limosa* (Say) from Shiverick Pond; 231 *Gyraulus deflectus* (Say), 140 *Physella*

heterostropha (Say), 2 *Lyogyrus pupoides* (Gould), 22 *Amnicola limosa* (Say), 340 *Helisoma antrosom?* (Conrad), from Sider's Pond; 145 *Pseudosuccinea columella* (Say), from Dump Pond; 500 *Physella heterostropha* from the Sandwich Fish Hatchery Pond; or in 500 *P. heterostropha* from Mill Pond, Sandwich.

DISCUSSION

At the present time there is no recognized schistosome dermatitis problem in eastern Massachusetts. Extensive collecting in various freshwater areas has failed to reveal substantial evidence that there may be such a problem in the future. For such a problem to exist in any area there must be present in fair numbers a definitive host or hosts for the schistosome cercariae, a sufficient number of intermediate hosts infected with cercariae, and then the area must be used by human beings for swimming, wading or trapping (any occupation wherein the susceptible individual's skin comes in contact with dermatitis-producing schistosome cercariae).

In many of the areas examined, the abundance of the proper snails has been markedly diminished or entirely eradicated by one or more of the following: (a) streams covered by oil for mosquito control or otherwise polluted by sewage, etc., (b) lakes and ponds prepared to serve as reservoirs (this entails building rock banks which prohibit the growth of water weeds, thereby resulting in a shortage of food for the intermediate and definitive hosts of the dermatitis producing schistosomes), (c) artificial, covered channels for brooks and streams, (d) ducks and rats in large enough numbers to markedly decrease the snail population by using the snails for food. In other areas where the proper snails are present, there has been an apparent disappearance of the definitive hosts. Where both the intermediate and definitive hosts are found present there exists a minimum of wading or swimming.

In the vicinity of the Muddy River and the Charles River Basin where Tanabe reported collecting *Stagnicola palustris* infected with schistosome cercariae in 1921 and 1922, this snail species no longer occurs. However, it is possible the snail or its eggs may be washed downstream from other areas at certain seasons of the year. No other reported intermediate host of *S. douthitti* was found in this or any other area. The only recognized definitive host found in the Muddy River area was the brown rat and Price (1931) and others have shown that the life cycle of *Schistosomatium douthitti* cannot be successfully completed in rats because the eggs fail to break through the lumen of the intestine.

Because snails in the various areas failed to reveal schistosome infections, concentrated efforts were not made to trap definitive hosts.

Marine gastropods were not collected in the present survey but it is possible that in certain marine swimming beaches or bays, they may be infected with dermatitis-producing schistosome cercariae in numbers sufficient to be a problem.

SUMMARY

1. A study has been made of five of Tanabe's preparations of *Schistosomatum pathlocopticum* and, on a basis of morphological and experimental comparison, the species demonstrated to be a synonym of *S. douthitti* (Cort, 1914), the cercariae of which are known to produce water itch or schistosome dermatitis.

2. Collections in the same areas where Tanabe (1923) found schistosome cercariae in *Stagnicola palustris* (Müller) failed to reveal the intermediate host. Examinations of 2781 *S. palustris* collected in other areas, failed to reveal infections of schistosome cercariae.

3. Extensive collecting of fresh water gastropods in 10 arbitrarily designated areas including waterways, lakes, and ponds where there is or could be swimming or wading in eastern Massachusetts, has failed to reveal a water itch problem. Examinations of 5837 *Physella heterostropha* (Say), 140 *Viviparus malleatus* (Reeve), 461 *Gyraulus parvus* (Say), 197 *Annicola limosa* (Say), 204 *Helisoma trivolvis* (Say), 373 *Galba humilis modicella* (Say), 85 *Lymnaea auricularia* (Linnaeus), 197 *Pseudosuccinea columella* (Say), 340 *Helisoma antrosomum?* (Conrad), 9 *Planorbula jenksii* Carpenter, 528 *Gyraulus deflectus* (Say), 108 *Galba umbilicata* C. B. Adams, 2 *Lyogyrus pupoideus* (Gould), from the various areas with one exception failed to reveal schistosome infections.

4. One *Physella heterostropha* (Say) collected, had an immature schistosome infection which was not of the *Schistosomatum* type but which was similar to *Cercaria physellae* Talbot, 1936, and other closely related species known to be dermatitis producers.

BIBLIOGRAPHY

- CORT, W. W. 1914 Larval trematodes from North American fresh-water snails. *J. Parasitol.* 1: 65-84.
 ——— 1917 Homologies of the excretory system of the forked-tailed cercariae. A preliminary report. *J. Parasitol.* 4: 49-57.
 ——— 1918 Adaptability of schistosome larvae to new hosts. *J. Parasitol.* 4: 171-175.
 ——— 1921 The development of the Japanese blood-fluke, *Schistosoma japonicum* Katsurada, in its final host. *Am. J. Hyg.* 1: 1-38.
 ——— 1936 Studies on schistosome dermatitis. I. Present status of the subject. *Am. J. Hyg.* 23: 349-371.
 CORT, W. W. AND TALBOT, S. BENTON 1936 Studies on schistosome dermatitis. III. Observations on the behavior of the dermatitis-producing schistosome cercariae. *Am. J. Hyg.* 23: 385-396.
 KATSURADA, F. 1914 Schistosomiasis japonica. *Centr. Bakt. I Abt. Orig.* 72: 363-379.

- MCLEOD, J. A. 1940 Studies on cercarial dermatitis and the trematode family Schistosomatidae in Manitoba. Can. J. Res. 18(D): 1-28.
- MILLER, E. L. 1936 Studies on North American cercariae. Illinois Biol. Monogr. 10: 265-370.
- MILLER, H. M. 1924 Studies on furcocercous cercariae. I. *Cercaria douthitti* Cort and *Cercaria echinocauda* O'Roke. Tr. Am. Micr. Soc. 43: 145-149.
- PENNER, L. R. 1938 *Schistosomatium* from the muskrat, *Ondatra zibethica*, in Minnesota and Michigan. J. Parasitol. 24 Suppl: 26.
- 1939 Experimental studies on *Schistosomatium douthitti* (Cort) in mouse, rat, muskrat, guinea pig, and snow-shoe hare. J. Parasitol. 25 Suppl: 8.
- 1940 Morphological and biological studies on certain helminths of the muskrat. Thesis. University of Minnesota. (Unpublished.)
- PORTER, ANNIE 1938 The larval trematoda found in certain South African Mollusca, with special reference to Schistosomiasis (Bilharziasis). Publ. South African Inst. Med. Res. 8: 1-492.
- PRICE, H. F. 1929 A new adult schistosome, *Schistosomatium douthitti* (Cort). J. Parasitol. 16: 103.
- 1931 Life history of *Schistosomatium douthitti* (Cort). Am. J. Hyg. 13: 685-727.
- RANKIN, J. S. 1939 Ecological studies on larval trematodes from western Massachusetts. J. Parasitol. 25: 309-328.
- TANABE, B. 1923 The life history of a new schistosome, *Schistosomatium pathlocopticum* Tanabe, found in experimentally infected mice. J. Parasitol. 9: 183-198.

PHARYNGOSTOMOIDES PROCYONIS N. G., N. SP. (STRIGEIDA) A TREMATODE FROM THE RACCOON IN NORTH CAROLINA AND TEXAS

REINARD HARKEMA

Zoology Department, North Carolina State College

During the examination of twenty-three specimens of the raccoon, *Procyon lotor lotor* (L.) from North Carolina in the fall and winter of 1939-40 and 1940-41, thousands of small strigeid trematodes were found in the small intestine of fourteen specimens. These appear to represent a new genus of the family DIPLOSTOMIDAE, subfamily ALARIINAE. Mr. Allen McIntosh and Dr. E. W. Price of the U. S. Bureau of Animal Industry kindly corroborated the diagnosis. Additional material from East Texas kindly lent by Dr. A. C. Chandler necessitated revision of this description which originally was made from young specimens. The name *Pharyngostomoides* is herewith proposed for the new genus with *P. procyonis* as the type species.

Pharyngostomoides n. g.

Generic diagnosis: Family Diplostomidae: subfamily Alariinae: Body small, forebody broader and longer than hind body; pseudosuckers present; prosthetic glands usually present; holdfast organ slightly longer than broad. Oral sucker weakly developed, approximately the same size as ventral sucker. Ovary in posterior part of forebody, anterior to and smaller than testes. Mehlis' gland and vitelline reservoir between ovary and testes at junction of anterior and posterior segments. Uterus extending into mid-portion of holdfast organ. Testes approximately equal in size, usually ovoid, smooth to irregular in outline situated side by side in posterior segment. Genital cone present; genital atrium small, dorsal, subterminal. A deep dorsal furrow present in young specimens.

Type species: *Pharyngostomoides procyonis* n. sp.

Pharyngostomoides procyonis n. sp.

(Figs. 1-10)

Description: Body small, 0.4-1.8 mm in length; forebody spinose, scoop-shaped with lateral and posterior margins folded ventrally, longer and broader than hindbody in relaxed specimens ($0.31-1.1 \times 0.29-0.82$ mm) often flexed dorsally; hindbody ($0.17-0.89 \times 0.19-0.63$ mm) more or less conical. Oral sucker terminal, slightly broader than long ($0.038-0.120 \times 0.046-0.150$ mm). Pseudosuckers well developed, incupped in young specimens, protruding ear-like in older ones; with numerous fine ducts opening into them connecting with paired masses of unicellular glands lying laterally between levels of pharynx and ventral sucker, sometimes meeting in midline dorsal to pharynx; these glands in some specimens stain deeply and constitute a striking character, but in others they appear unstained and refractile, and are sometimes barely discernible. Ventral sucker ($0.058-0.120 \times 0.66-0.140$ mm) approximately same size as oral sucker; never raised above surface of body; between anterior margin of holdfast organ and intestinal fork; usually partially covered by holdfast organ or completely obscured by it. Holdfast organ well-developed (0.140-

Received for publication, April 27, 1941.

0.410×0.145–0.400 mm) slightly longer than broad; surface directed antero-ventrally with longitudinal slit-like opening in center. Prepharynx very short; pharynx (0.033–0.095×0.029–0.080 mm) usually contiguous with oral sucker; esophagus short, bifurcation anterior to ventral sucker; ceca extend to behind testes, gradually passing from dorsal side of forebody to ventral side of hindbody. Vitellaria follicular, distributed in forebody from acetabulum posteriorly to level of anterior margins of testes; slightly penetrate holdfast organ; in lateral margins extend to beyond level of acetabulum; not continuous ventrally in posterior region in older specimens (Fig. 9).

Testes variable in position, shape and size (Fig. 10); usually situated side by side in anterior part of hindbody but in 8 or 10 specimens of several hundreds examined either testis may be displaced backward; usually contiguous or close together but occasionally separated by width of testis; shape varies from spherical to long oval; size varies 0.128–0.385 mm in length and 0.089–0.240 mm in width. Right vas efferens arises from the medio-ventral surface of right testis, left vas efferens arises from the medio-anterior surface of its testis; vasa efferentia enter separately posterior surface of seminal reservoir; latter a confused mass of coils in front of ovary and between it and Mehlis' gland. Vas deferens arises from ventral side of reservoir and proceeds posteriorly between ventro-medial surfaces of opposite testes to behind latter where it becomes convoluted to form seminal vesicle; latter discharges usually through muscular ejaculatory pouch which is conspicuous in some specimens but not in others; ejaculatory duct unites with descending limb of uterus at base of genital cone. Latter often protruding beyond surface of body, thus obliterating greater part of small genital atrium. Cirrus protrusible.

Ovary small, reniform from ventral view, transversely elongated (0.035–0.120×0.078–0.190 mm), situated dorsally in posterior part of forebody. Oviduct arises from dorso-posterior side of ovary, extending posteriad, receives common vitelline duct upon entrance into postero-dorsal side of Mehlis' gland. Laurer's canal present; oviduct ampulla-like at its junction. Mehlis' gland and vitelline reservoir between ovary and testes often contiguous with anterior median borders of testes. Oviduct pursues sinuous course through Mehlis' gland emerging as ascending limb of uterus which continues anteriorly past ventral surface of ovary to mid-portion of holdfast organ. Descending limb passes posteriad in mid-ventral plane to unite with more dorsal ejaculatory duct and empties with latter at tip of genital cone. Vitelline ducts extend posterior-mesad to ventral surface of vitelline reservoir; latter extends ventro-dorsad discharging on its dorsal side by common vitelline duct into posterior end of oviduct.

In young specimens a deep crescentic furrow present just anterior to genital cone on dorsal side; in older specimens this furrow becomes part of genital atrium. Eggs 1 to 35 in number, measuring 0.082–0.093×0.055–0.060 mm. Excretory pore terminal. Nerve strand on each side extends posteriad from region of pharynx, lateral to intestinal ceca.

Host: Procyon lotor lotor (Linnaeus).

Habitat: Small intestine.

Locality: Wake County, Raleigh, North Carolina; Martin and Pasquotank Counties, North Carolina; Angelina County, Texas.

Specimens: U. S. Nat. Mus. Helm. Coll. Nos. 44850 (Type) and 44851 (Paratypes) of R. Harkema. Also Nos. 44932 and 44933 of A. C. Chandler.

Comparatively few strigeids have been reported from animals of the family PROCYONIDAE. La Rue and Townsend (1927) described *Alaria nasuae* from *Nasua nasica* and later mentioned (1932) that an unidentified strigeid had been obtained from the raccoon. Morgan and Waller (1940) reported a case of severe parasitism of a raccoon. Several different species of parasites were found including several specimens of *Fibri-*

cola cratera (Barker and Noll, 1915). *Fibricola laruei* from the raccoon in Canada was described by Miller (1940). Chandler (1942) found *Fibricola texensis* Chandler, 1942, infecting raccoons in east Texas.

The combination of prosthetic glands, opposite testes and dorsal furrow justify the erection of a new genus. The variability of these characters possibly may militate against this viewpoint. However, only a very few specimens out of hundreds examined showed any marked shifting of the testes. The conspicuousness or obscurity of the prosthetic glands suggests the possibility of variation in the metabolic activity of these glands and their development may be influenced by the age of the flukes. These glands and the dorsal furrow show similarities with *Sphinctero-diplostomum musculosus* where the pseudosuckers present the same appearance as in *Pharyngostomoides procyonis* but the glands are more diffuse. The crescentic dorsal furrow is represented by a tubular invagination with a sphincter.

Dubois (1938) lists the genera *Alaria*, *Cynodiplostomum*, *Fibricola*, *Pharyngostomum*, *Podospathalum* as members of the sub-family ALARIINAE. *Pharyngostomoides* possesses characters which separate it from these genera. It obviously is different from the aberrant *Podospathalum*. It can be separated from *Cynodiplostomum* by its smaller size, ventrally folded posterior margin of forebody, presence of a prepharynx and esophagus, prosthetic glands, distribution of vitellaria, relatively larger holdfast organ, and in the position of ovary and testes, *Fibricola* and *Alaria* both have the testes arranged tandemly; the former possesses no pseudosuckers and in *Alaria* the holdfast organ is more elongate and there are no glands. *Pharyngostomum* is the only previously described genus of ALARIINAE which has opposite testes in the hindbody. Its very large pharynx, very large piriform holdfast organ, absence of pseudosuckers and larger size validate its separation from *Pharyngostomoides*.

BIBLIOGRAPHY

- CHANDLER, A. C. 1942 The morphology and life cycle of a new strigeid, *Fibricola texensis*, parasitic in raccoons. Tr. Am. Micr. Soc. 61:
DUBOIS, G. 1938 Monographie des Strigeida (Trematoda). Mem. Soc. Neuchatel. Sc. Nat. 6: 1-535.
LA RUE, G. R. AND E. W. TOWNSEND 1927 Studies on the trematode family Strigeidae (Holostomidae). *Alaria nasuae* sp. nov. J. Parasitol. 14: 124.
——— 1932 A morphological study of *Alaria nasuae* La Rue and Townsend (Trematoda: Alariidae). Tr. Am. Micr. Soc. 51: 252-263.
MILLER, M. J. 1940 A new trematode, *Fibricola laruei*, from the raccoon in Canada. Canad. J. Res. 18 (D): 333-335.
MORGAN, B. B. AND E. F. WALLER 1940 Severe parasitism in a raccoon (*Procyon lotor lotor*, Linnaeus). Tr. Am. Micr. Soc. 59: 523-527.

EXPLANATION OF PLATES

Pharyngostomoides procyonis n. g., n. sp.

ABBREVIATIONS

cvd—common vitelline duct	rvd—right vitelline duct
ejp—ejaculatory pouch	sr—seminal reservoir
exp—excretory pore	sv—seminal vesicle
gc—genital cone	tr—right testis
hf—holdfast organ	uta—ascending limb of uterus
int—intestinal cecum	utd—descending limb of uterus
lc—Laurer's canal	vd—vas deferens
lvd—left vitelline duct	vel—left vas efferens
mg—Mehlis' gland	ver—right vas efferens
oot—oötype	vit—vitelline follicle
ov—ovary	vr—vitelline reservoir
ovd—oviduct	

PLATE I

FIG. 1. Camera lucida drawing of whole mount of young specimen, ventral view.

FIG. 2. Camera lucida drawing of cross section through middle of holdfast organ, anterior view, ventral side uppermost.

FIG. 3. Reconstruction of male reproductive system as seen from left side in sagittal view. Left testis not shown.

FIG. 4. Partial reconstruction of cross sections at level of seminal reservoir, showing extent of Mehlis' gland, seminal reservoir, entrance and ventral limits of vasa efferentia and exit of vas deferens, descending limb of uterus and intestinal ceca. Posterior view, dorsal side uppermost.

FIG. 5. Camera lucida drawing of cross section through middle of hindbody.

FIG. 6. Reconstruction of female reproductive system as seen from left side in sagittal view. Right testis shown, right vitelline duct not shown.

FIG. 7. Reconstruction of cross sections at level of vitelline reservoir and Mehlis' gland showing oviduct, entrance of Laurer's canal and common vitelline duct, oötype, Mehlis' gland, vitelline ducts, vitelline reservoir and vitellaria. Anterior aspect, dorsal side uppermost.

PLATE II

(Drawn by A. C. Chandler)

FIG. 8. Ventral view of older specimen.

FIG. 9. Specimen showing arrangement of vitellaria and extruded cirrus.

FIG. 10. Variety of sizes and shapes observed.

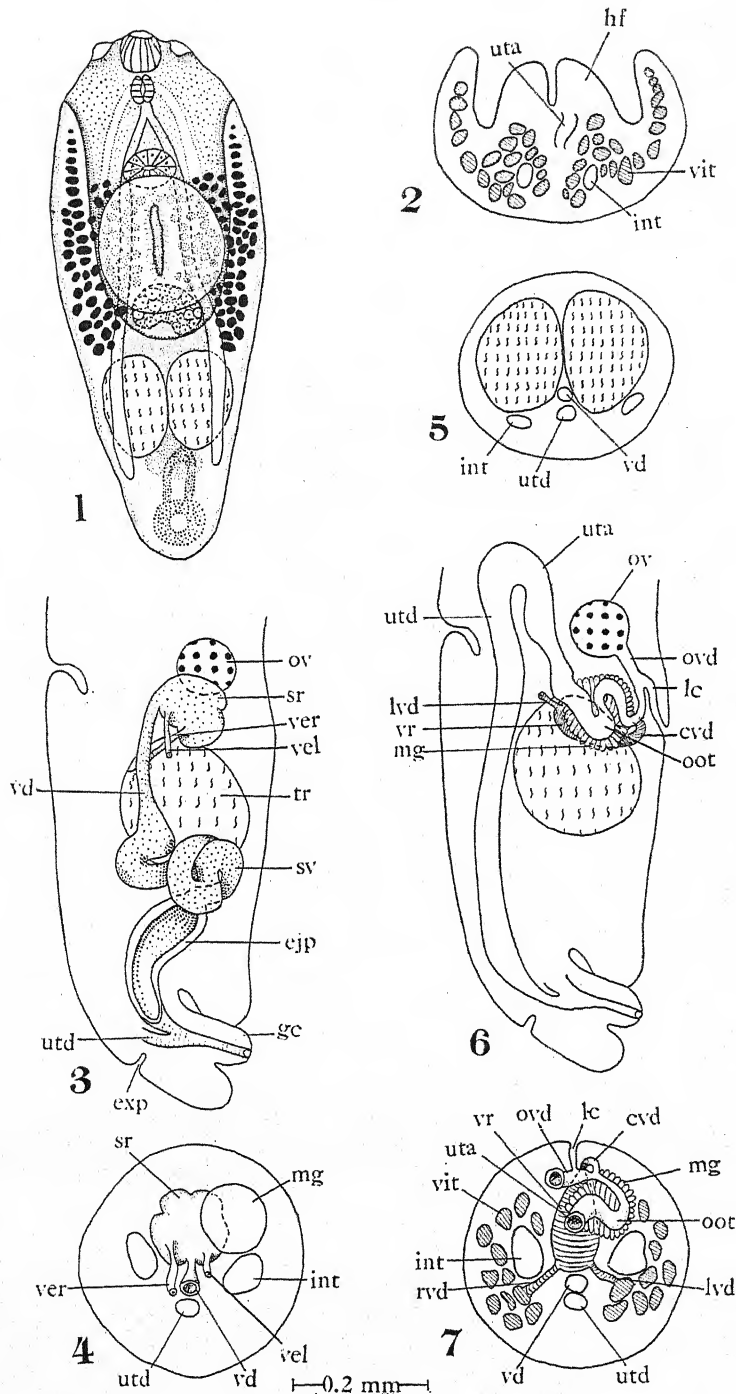


PLATE I

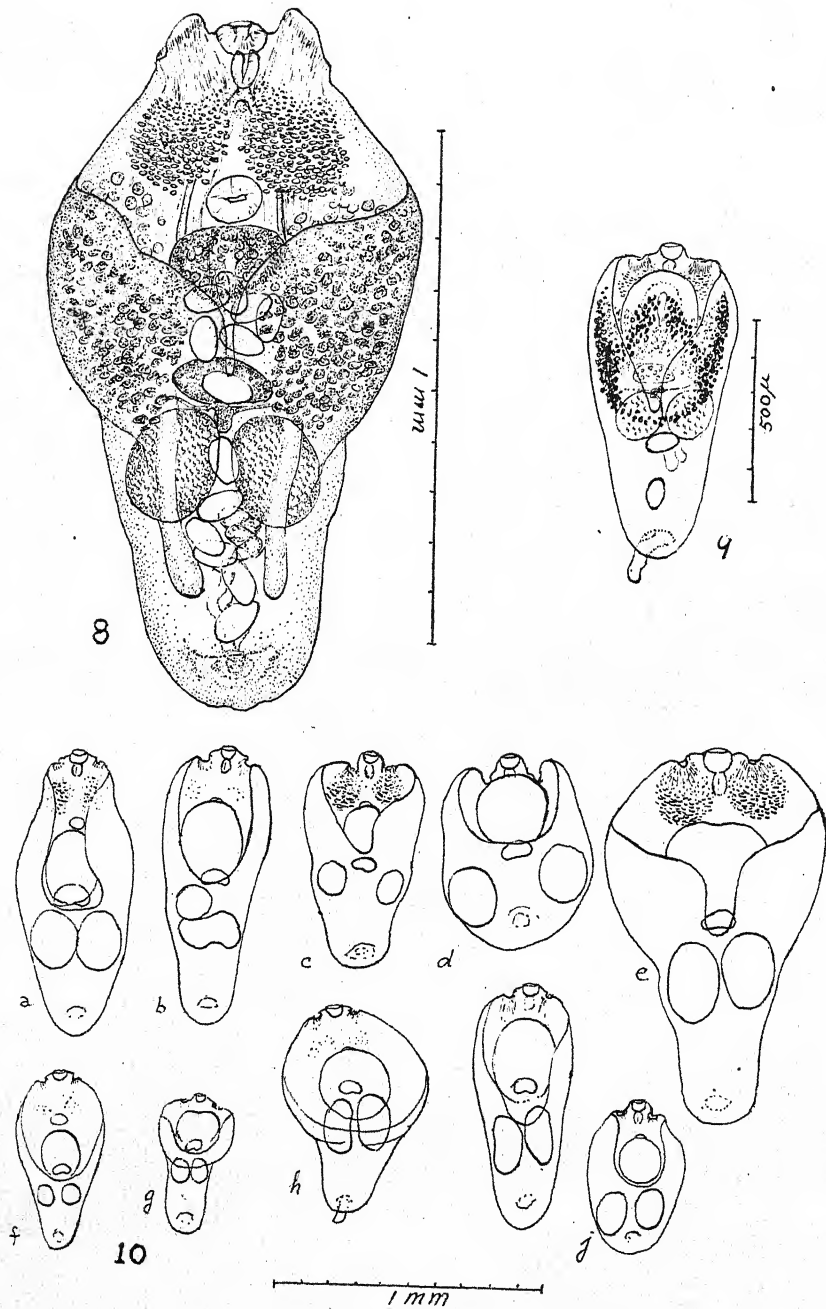


PLATE II

THE BIOLOGY AND CULTURE OF *NEOAPLECTANA*
CHRESIMA, A NEW NEMATODE
PARASITIC IN INSECTS¹

R. W. GLASER, E. E. MCCOY, AND H. B. GIRTH

A few years ago a number of corn earworms (*Heliothis armigera*) near Moorestown, New Jersey, were found dead and dying from a condition which on examination proved to be due to nematode parasitism.² Later the present writers found the same nematode parasitic in Japanese beetle (*Popillia japonica*) larvae. From 1937 to 1940 this parasite was found among the beetle larvae in 14 places distributed among 9 counties of New Jersey and in one locality in Maryland. In all these places this nematode seemed to represent the only helminth parasite present and was apparently causing a considerable reduction in the host population. Specimens of the nematode were submitted to G. Steiner of the Bureau of Plant Industry, United States Department of Agriculture, who stated that the form represented a new species of the genus *Neoaplectana*. He will shortly publish a description of this and other new species and a key to all known forms of the genus *Neoaplectana*. Steiner furnishes the following diagnosis which is published here with his permission, the name of the species being derived from the Greek word *χρήσιμα* meaning useful.

Neoaplectana chresima Steiner

"*Diagnosis*: *Neoaplectana* resembling *N. glaseri* Steiner, 1929 (1), but the adults of both sexes different by a sharply set off, often mucronate, tail terminus, by an excretory pore opening much closer cephalad, i.e., at a latitude near the middle of the corpus of the esophagus or at about one-third of the total length of the esophagus. Spicula proximally capitate and curved outward. Arrangement of male copulatory papillae somewhat different. Larvae with four equidistant longitudinal striae on lateral fields, tail elongate-conical, terminus not set off, the phasmids located near middle.

"*Type host*: *Heliothis armigera* Hbn.

"*Type locality*: Moorestown, N. J., U. S. A."

Our studies showed the life cycle of *N. chresima* to be similar to that of *N. glaseri* (2). The adults of *N. chresima* are somewhat like *N. glaseri*

Received for publication, April 30, 1941.

¹ A study conducted cooperatively by the Rockefeller Institute for Medical Research, Princeton, N. J., the New Jersey Department of Agriculture, and the United States Department of Agriculture.

² On November 26, 1934, Dr. W. H. Larrimer of the Bureau of Entomology and Plant Quarantine collected three diseased pupae of the corn earworm (*Heliothis armigera* Hbn.) from the soil in an outdoor hibernation cage at Moorestown, New Jersey. These were examined by Dr. Vera K. Charles of the Bureau of Plant Industry, who found them to contain a large number of nematodes and transmitted the material to Dr. G. Steiner of that Bureau for further examination. Steiner pronounced them to be representatives of a new species of the genus *Neoaplectana*.

but smaller. As in the latter species the females are ovoviviparous, but each produces between 250 and 400 young in contradistinction to *N. glaseri*, which generally produces approximately 15 young. (Certain rare "giant" females of *N. glaseri* may produce as many as 1400 young (3).) At first it was thought that *N. chresima* females were oviparous as well as ovoviviparous because free embryonated ova were often found. After careful study, however, it was concluded that while such free embryonated ova may develop normally they were derived from mature females that ruptured in a non-isotonic solution. In an isotonic solution free ova are not obtained, and *N. chresima* females then give birth only to living young. The larval forms are smaller than the corresponding stages in *N. glaseri*. The infective stage and the adults are more sluggish. The second-stage *N. chresima* larvae ensheath more rapidly than is the case with *N. glaseri*. Dead Japanese beetle larvae parasitized by *N. chresima* rarely smell badly, and when they do the parasites are usually dead. In general, the grub cadavers assume a dirty dull yellow color in contradistinction to an ochreous brown color of those parasitized by *N. glaseri*. In the former case the larval contents become quite viscid and of a dirty yellow hue. In the latter the contents are more fluid and of a clear brown color. When Japanese beetle larvae are experimentally exposed to soil or food contaminated with *N. chresima* they die in about 4 days.

Other hosts experimentally infected with *N. chresima* were the grasshopper (*Melanoplus* sp.), the European corn borer (*Pyrausta nubilalis*), the corn earworm (*Heliothis armigera*), and catalpa sphinx caterpillars (*Ceratonia catalpa*). In one of these experimentally infected catalpa sphinx caterpillars the nema reproduced to such an extent that at death 1,140,000 second-stage larvae were counted.

It was important to know whether *N. chresima* could be cultured on artificial media as is the case with *N. glaseri* (2). Occasionally *N. chresima* would show slight development in some particular medium, but it could not be carried through a second transplant. Such cultures often smelled badly, and it was assumed that bacteria were responsible for the failures. Second-stage larvae of *N. chresima* were then subjected to a sterilizing process described in 1940 (4, 5). After the nemas were bacteria-free they grew well on pieces of fresh, sterile rabbit kidney and survived in transplants.

The method was the following. A piece of kidney weighing one or two grams is placed in the water of condensation at the bottom of an ordinary nutrient agar slant after which the surface of the tissue is inoculated with the nematodes. To prevent evaporation the cotton plug of the tube is trimmed and pushed down, and the surface covered with sealing wax. By means of a hot wire a small perforation is later made through the hardened wax. All tubes are held in a slanted position and

incubated at room temperatures, 22–28° C. The nutrient agar is not a necessary nutritional constituent. Kidney alone suffices, but under the conditions described above the slanted agar affords a surface upon which the nematodes can migrate when they are impelled to do so. Some of these sterile cultures of *N. chresima* have been maintained for about two years and are to date in their 28th transplant. Transplants may be made at intervals of one to three months.

Recently an autoclaved medium has been devised which gives excellent growth and, because it can be more cheaply prepared in larger amounts, has replaced the older fresh rabbit tissue method. This medium is a semisolid gel at pH 7.0, in the proportion of 20 gm ground beef kidney or liver, 100 ml water, 0.5 gm sodium chloride, and 0.5 gm agar. This is autoclaved and may be stored for two weeks prior to use. It may be used for *N. glaseri* as well as for *N. chresima*, although it is not the substrate which is usually employed for the former species. Since both species are aerobic they develop on and near the surface of the semisolid gel. This gel offers enough resistance so that the nemas do not perish by sinking and further obtain enough foothold, so to speak, to effect their molts. Every two days the flasks containing the cultures are gently shaken to bring fresh nutrients from the lower layers to the surface. Under these conditions the cultures ordinarily reach stability in about 15 to 30 days, depending upon the amount of medium, the number of nemas initially used as inoculum, and fluctuations in the daily temperatures. When the cultures have reached stability no further increase in numbers is obtained on longer incubation and the dominant form is the infective larva.

A comparative count on the above medium for *N. glaseri* and *N. chresima* follows. Fifty ml of medium were used throughout, and the flasks were initially inoculated with approximately 10,000 infective forms of *N. glaseri* or *N. chresima* respectively. The final counts (recorded below as the average of 5 separate samples) were made in 23 days, at which time these cultures had reached stability. The cultures were free of all microorganisms, as comprehensive sterility tests showed.

Nutrient in medium	Final count for <i>N. glaseri</i>	Final count for <i>N. chresima</i>
Beef liver	538,000	522,000
Beef kidney	428,000	386,000
Beef muscle	18,000	6,000

The above test shows that the two species grew well on both liver and kidney but not at all on beef muscle. The counts on the last nutrient, within the experimental error, indicate no increase above the number initially introduced.

In 1940 (5) the importance of sterility was emphasized in rearing *N.*

glaseri to obtain the maximum yield. As regards *N. chresima*, no growth is ordinarily obtained when contaminants are present. However, in the presence of one bacterium a number of strains of *N. chresima* have developed and have consistently yielded cultures on transplantation. This contaminant is a nonputrefactive, nonsporulating, gram negative, motile bacillus originally derived from the soil. Cultures of *N. chresima* associated with this seemingly innocuous bacillus are not so luxuriant as are the sterile cultures. The data below illustrate this point. The experiments were performed as previously described except that a smaller amount of medium and a smaller nematode inoculum were used.

Final counts for *N. chresima* cultures
(Nutrient in medium is beef liver.)

Strain No.	Sterile	Contaminated with one bacterial species
1	266,000	199,000
2	280,000	14,000
3	300,000	86,000

The sterile cultures of *N. chresima* have now been carried by transplantation for two years, and those contaminated with the one bacillus for one and one-half years. For future physiological studies, an attempt is now being made to adapt *N. chresima* to liquid nutrients such as the nutrient solution for *N. glaseri* described in 1940 (5).

No field tests, to appraise the economic possibilities of *N. chresima*, have as yet been attempted.

SUMMARY

A new nematode, preliminarily described by Steiner as a new species, *Neoaplectana chresima*, was found naturally parasitic in two species of insect hosts, and five additional species of insects were found to be susceptible to experimental infection. Certain biological differences between *N. chresima* and *N. glaseri* are briefly outlined. The sterile culture of *N. chresima* is described and the deleterious effects of contaminants emphasized.

BIBLIOGRAPHY

1. STEINER, G. 1929 *Neoaplectana glaseri* n. gen., n. sp. (Oxyuridae), a new nematode parasite of the Japanese beetle (*Popillia japonica* Newm.). J. Wash. Acad. Sc. 19: 436.
2. GLASER, R. W., MCCOY, E. E. AND GIRTH, H. B. 1940 The biology and economic importance of a nematode parasitic in insects. J. Parasitol. 26: 479.
3. MCCOY, E. E., GIRTH, H. B. AND GLASER, R. W. 1938 Notes on a giant form of the nematode *Neoaplectana glaseri*. J. Parasitol. 24: 471.
4. GLASER, R. W. AND STOLL, NORMAN R. 1940 Exsheathing and sterilizing infective nematode larvae. J. Parasitol. 26: 87.
5. GLASER, R. W. 1940 The bacteria-free culture of a nematode parasite. Proc. Soc. Exper. Biol. and Med. 43: 512.

SOME MOSQUITO HOSTS TO AVIAN PLASMODIA WITH
SPECIAL REFERENCE TO *PLASMODIUM*
*GALLINACEUM*¹

PAUL F. RUSSELL AND BADRI NATH MOHAN

Brumpt (1935) described *Plasmodium gallinaceum* and later (1936 a, b) reported experimental transmission to fowls and certain other birds by *Aedes aegypti* and *Aedes albopictus* ("albopicta"). No development of the parasite occurred in *Culex fatigans*. Roubaud et al (1939) reported complete development of this plasmodium in *Aedes geniculatus*, a tree-hole breeder of France.

Huff (1932) reviewed the literature in reference to mosquitoes found susceptible to avian malaria, irrespective of *Plasmodium* species (excluding, of course, *P. gallinaceum*) and compiled the following list:

Culex (Culex) quinquefasciatus Say
Culex (Culex) pipiens Linn.
Culex (Culex) hortensis Ficalbi
Culex (Culex) territans Walker
Culex (Culex) salinarius Coq.
Culex (Culex) tarsalis Coq.
Aedes (Stegomyia) aegypti Linn.
Aedes (Ochlerotatus) mariaae Ed. Serg.
Aedes (Ochlerotatus) communis De Geer
Aedes (Finlaya) triseriatus Say
Culicella longiareolata Macq.
Anopheles (Mysomyia) subpictus Grassi

Nono (1932) added *Lutzia fuscana* Wied. [*Culex (Lutzia) fuscana* Wied.] to this list. Reichenow (1932) demonstrated the susceptibility of *Theobaldia annulata*. Herman (1938) added *Aedes sollicitans* and *Theobaldia melaneura*.

We have found the following species to be suitable hosts for *P. praecox*, of local sparrows, in our laboratory:

Culex (Culex) bitaeniorhynchus Giles, 1901
Culex (Culex) gelidus Theo., 1901
Culex (Culex) whitmorei (Giles) 1904
Culex (Culex) theileri Theo., 1903

The chief purpose of this note is to record the development of *P. gallinaceum* in several other species of mosquitoes found in the Nilgiris area of Madras Presidency, India. The strain of *P. gallinaceum* used origi-

Received for publication, May 14, 1941.

¹ These studies were made under the auspices and with the support of the International Health Division of The Rockefeller Foundation co-operating with the Pasteur Institute of Southern India at Coonoor, Nilgiris, and with the Madras Provincial Health Department.

nated in Ceylon and was sent to the authors by courtesy of Lt.-Col. H. E. Shortt, I.M.S., Director of the King Institute of Preventive Medicine, Guindy, Madras. The infections in these mosquitoes were developed in connection with a study of sporozoite agglutination reported elsewhere (Mulligan et al, 1940; Russell et al, 1941).

Complete exogenous development of *P. gallinaceum* has been observed by us in the following species fed on infected domestic fowls:²

Armigeres (Armigeres) obturbans (Walk.) 1860.

Breeds mostly in cut bananas, but also in tree holes, cut bamboos, and domestic water, often very foul.

Armigeres (Armigeres) kuchingensis Edwards, 1915.

Breeds in cut bananas and bamboos.

Armigeres (Armigeres) aureolineatus Leicester, 1908.

Breeds in cocoanut shells.

Aedes (Stegomyia) aegypti (Linn.) 1762.

Breeds in domestic water in artificial containers.

Aedes (Stegomyia) albopictus (Skuse) 1894.

Breeds in tree holes, cut bamboos and bananas, cocoanut shells, and rarely in domestic water.

Aedes (Stegomyia) unilineatus (Theobald) 1906.

Breeds in tree holes.

Aedes (Stegomyia) pseudalbopictus Borel, 1928.

Breeds in cut bamboos, cocoanut shells.

Aedes (Stegomyia) vittatus (Bigot) 1861.

Breeds in tree holes, rock pools, cocoanut shells, and domestic water.

Aedes (Stegomyia) scutellaris (Walker) 1859.

Breeds in tree holes, cocoanut shells, and domestic water.

Aedes (Finlaya) pseudotaeniatatus (Giles) 1901.

Breeds in tree holes, rock pools, domestic water.

Aedes (Finlaya) chrysolineatus (Theobald) 1907.

Breeds in tree holes, cut bamboos, rock pools, and domestic water.

Aedes (Finlaya) pallirostris Edwards, 1922.

Breeds in rock pools and tree holes.

(Specific names from Barraud, 1934).

We have failed in repeated attempts to obtain development of *P. gallinaceum* in the following species:

Culex (Culex) fatigans Wiedemann, 1828.

Culex (Culex) mimeticus Noe, 1899.

Culex (Culex) bitaeniorhynchus Giles, 1901.

Anopheles stephensi Liston, 1901.

Anopheles jeyporiensis James, 1902.

All mosquitoes after feeding on infected fowls were kept in an insectary where temperature was maintained between 70° and 85°F and relative humidity between 75 and 85 per cent. Sporozoites in all susceptible species were seen after incubation periods of from 9 to 12 days.

Four species, *Armigeres obturbans*, *Armigeres kuchingensis*, *Aedes albopictus*, and *Aedes aegypti*, were found most suitable for the develop-

² Our diagnoses of species have been confirmed by courtesy of Dr. I. M. Puri, Entomologist, Malaria Institute of India, Delhi.

ment of very heavy sporozoite infection of salivary glands. The *Armigeres* species would feed on fowls at any time during daylight but especially well in the afternoon. The *Aedes* species fed most avidly in the morning but would also feed at other times, even at night. Insectary colonies of *obturbans* and *albopictus* have been maintained without difficulty.

BIBLIOGRAPHY

1. BARRAUD, P. J. 1934 The Fauna of British India, Diptera, Vol. V. Family Culicidae. Tribes Megarhinini and Culicini. London. Taylor and Francis. 463 pp.
2. BRUMPT, E. 1935 Paludisme aviaire: *Plasmodium gallinaceum* n. sp. de la poule domestique. Compt. Rend. Acad. Sc. 200: 783-785.
3. ——— 1936a Réceptivité de divers oiseaux domestiques et sauvages au parasite (*Plasmodium gallinaceum*) du paludisme de la poule domestique. Transmission de cet hématozoaire par le moustique *Stegomyia fasciata*. Compt. Rend. Acad. Sc. 203: 750-752.
4. ——— 1936b Étude expérimentale du *Plasmodium gallinaceum*, parasite de la poule domestique. Transmission de ce germe par *Stegomyia fasciata* et *Stegomyia albopicta*. Ann. Parasitol. 14: 597-620.
5. HERMAN, C. M. 1938 Mosquito transmission of avian malaria parasites (*Plasmodium circumflexum* and *Plasmodium cathemerium*). Am. J. Hyg. 27: 345-350.
6. HUFF, C. G. 1932 Further infectivity experiments with mosquitoes and bird malaria. Am. J. Hyg. 15: 751-754.
7. MULLIGAN, H. W., RUSSELL, P. F., AND MOHAN, B. N. 1940 Specific agglutination of sporozoites. J. Malar. Inst. India. 3: 513-524.
8. NONO, A. M. 1932 Avian malaria studies. VI. Susceptibility of *Lutzia fusca* (Wiedemann) Edwards to avian malaria. Philippine J. Sc. 49: 225-229.
9. REICHENOW, E. 1932. Die Entwicklung von *Proteosoma circumflexum* in *Theobaldia annulata* nebst Beobachtungen über das Verhalten anderer Vogelplasmodien in Mücken. Jenaische Z. Naturw. 67: 434-451.
10. ROUBAUD, E., COLAS-BELCOUR, J., AND MATHIS, M. 1939 Transmission de *Plasmodium gallinaceum* par *Aedes geniculatus*. Bull. Soc. Path. Exot. 32: 28-30.
11. RUSSELL, P. F., MULLIGAN, H. W., AND MOHAN, B. N. 1941 Specific agglutination properties of inactivated sporozoites of *P. gallinaceum*. J. Malar. Inst. India. 4: 15-24.

A SIMPLE APPARATUS FOR DETERMINING THE VIABILITY OF EMBRYONATED HELMINTH OVA

ROBERT L. BUTLER, JR., AND REED O. CHRISTENSON

Zoology-Entomology Department, and the Alabama Agricultural Experiment Station, Auburn, Alabama

One of the major problems confronting workers in making studies on the effects of such agencies as chemicals, heat, cold, ultra-violet radiation and ultra-short radio waves (Hertzian waves) on helminth ova has been the application of a suitable test for viability. It is easy to subject unembryonated eggs to chemical and physical variants and to use subsequent development as indicative of life. If the ova are embryonated, on the other hand, the problem is more complicated, since the only suitable test is motility. Without some external stimulation the encapsulated larvae may remain quiescent for long periods causing the observer to spend many hours at the microscope before an accurate pronouncement can be made. This is especially true in longevity experiments during which the natural activity of the embryo decreases with the increase in the time of incubation. The method described below activated eggs of *Heterakis gallinae* and *Ascaridia galli* from cultures nearly two years old, and allowed making a rapid determination of viability on a large series.

Before the development of the apparatus described below, various unsatisfactory methods were used in attempting to stimulate activity on the part of embryonated eggs; including electric shock, exposure to strong light rays, and placing the eggs in an electric oven before preparing them on slides for observation. The results were so variable as to render these methods unreliable. A modification of the Lamson-Brown (1938) technique was then tried, with slide preparations being placed over a beaker containing steaming-hot water. This gave fair results, but the condensation of water on the slide required wiping it; often the ova were killed by too intense heat, and very often the larvae, if stimulated to activity by the process, apparently returned to quiescence before observation was made.

The method which gave the basic idea for the apparatus described below produced excellent results, activating larvae to observable motion even in the oldest cultures in the laboratory. A piece of copper wire about six inches long was coiled at one end, the coil being somewhat less in diameter than the aperture in the stage of the microscope and open in the center to admit the light from the condenser. The wire was then bent at an obtuse angle about one inch from the coil, and the coil angled

in the opposite direction to the same degree. The instrument thus formed had an open sigmoid shape, the wire handle and heating coil being parallel. Slide preparations were placed on the microscope, a suitable ovum selected microscopically, and the condenser lowered about one inch, the light being adjusted to give good visibility. The coil was then heated over a burner until red and applied through the stage aperture to the slide.

Even though heating the ovum from below with a hot wire gave the desired results there were some obvious difficulties to the method. Often

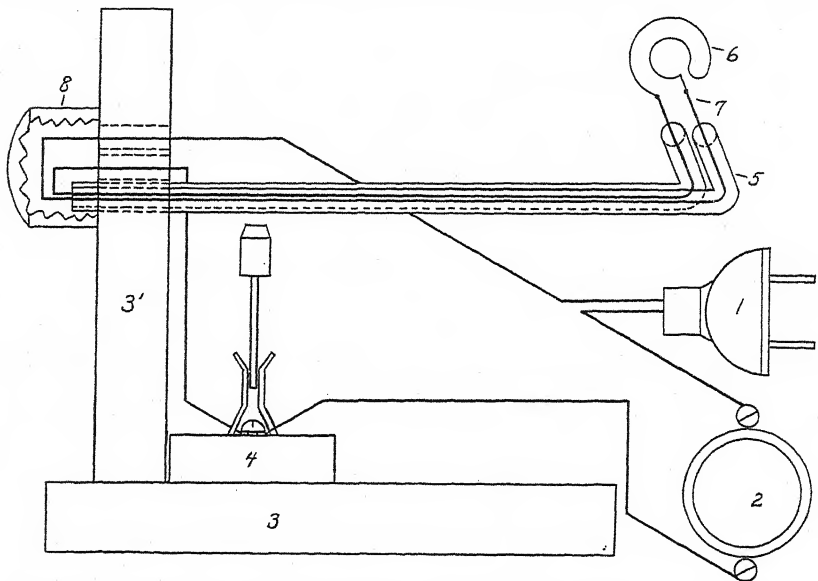


FIG. A. Apparatus to determine the viability of helminth eggs. Side view. (1) Electric cord plug; (2) Socket for 150-Watt bulb; (3, 3') Wooden support; (4) Knife-blade switch; (5) Glass tube supports of the heating element; (6) High resistance heating wire; (7) Copper wire leads to heating element; (8) Celluloid cap over the naked wire junctions.

the hot wire came into contact with the stage, and occasionally the glass parts of the microscope were touched. Sometimes the slide dried rapidly preventing sufficient observation. To obviate these difficulties a simple electric heating element was devised (Figs. A and B) which gives excellent results, viable embryos being stimulated to activity in a very short time. The apparatus is cheap in construction, and saves many hours of tedious observation when a large series of cultures is to be tested.

The equipment needed is about 5 feet of ordinary electric light cord attached to a socket plug; a 150-Watt standard bulb and a socket; 3 feet of #18 copper wire; a few inches of #30 high resistance wire for the heating element; a standard knife-blade switch; two pieces of thick-

walled, high melting-point glass tubing with an inside diameter of 1 mm; two blocks of wood to serve as a support, and the necessary screws to attach the socket and switch to their respective supports.

The socket with the 150-Watt light bulb and the switch are connected in series to the light cord. The two glass rods are cut into lengths of $10\frac{1}{2}$ inches and bent at right angles about $3\frac{1}{2}$ inches from the end. Careful, uniform heating of the tubes must be applied so as not to reduce the inside diameter in the bent area. Two lengths of copper

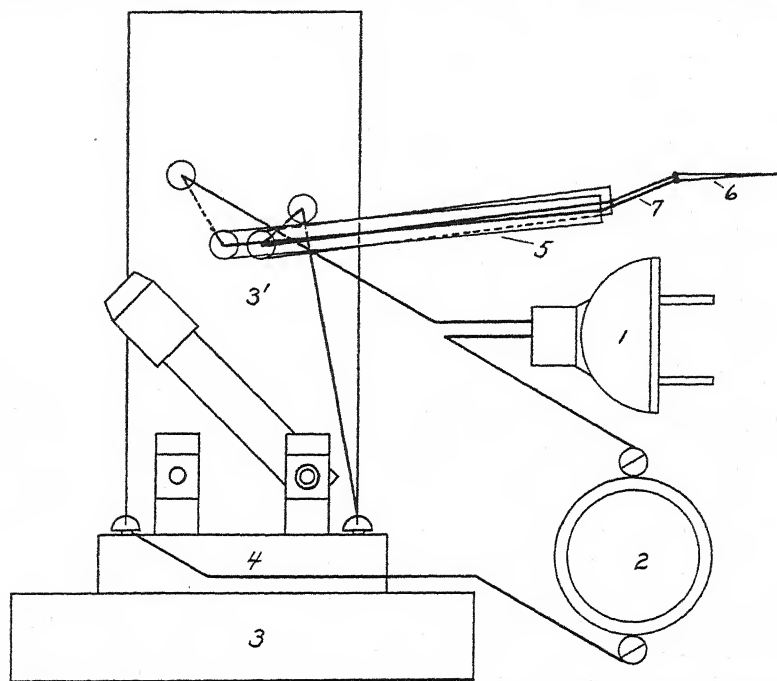


FIG. B. Front view of viability testing apparatus showing the heating element (6) in position. The parts of the apparatus are numbered as in Fig. A. The wiring is indicated by heavy lines.

wire are cut, each 12 inches in length, and the insulation removed. These are threaded through the glass tubes from the long end. Since the wire used nearly fills the glass conduit it must be worked through slowly, with constant twisting, until about $\frac{1}{2}$ inch projects from the short end from the angle. The high resistance wire (about 2 inches long) is then soldered into place on the protruding ends of the copper wire using acid core solder, the two copper wires thus being joined, and insulated in glass. The glass rods are inserted at the desired height by the long end from the angle into the upright of the stand formed from the two wooden blocks. By careful choice of bits for boring the holes a snug fit for the glass tubes results so that no additional support is required. The free

wires projecting from the back of the upright are soldered into the circuit and the junctions insulated with tape. In the case of the instrument figured the celluloid covering of the cork of a chemical bottle was taped to the support over the naked wires. When the circuit has thus been completed the high-resistant wire is moulded into a coil of the desired diameter, care being taken so that the wire loops in the heating element thus formed do not touch. The copper poles to which the coil is attached are bent so that the coil lies parallel to the stage in the desired position in the stage aperture, the wooden support being located at the side of the microscope. By simply closing the circuit with the key, the heating coil becomes radiant and the observer can watch the reactions of the embryo in the egg even under the high powers of the microscope. At the first signs of motility, observable in our tests in less than a half minute, the switch is opened. The amount of heat supplied can be governed by the size of the bulb in the circuit. The larger the bulb, the greater the amount of heat radiation. A 150-Watt bulb will give sufficient heat without causing the heating unit to become red, thus reducing the period of its effectiveness. Fig. A shows a side view of the apparatus, and Fig. B the front view.

HELMINTHS OF TREE SQUIRRELS IN SOUTHEAST TEXAS

ASA C. CHANDLER

Biological Laboratory, Rice Institute, Houston, Texas

In cooperation with Mr. Rollin H. Baker, Field Biologist of the Texas State Game, Fish and Oyster Commission, engaged in ecological studies of native fur animals in southeast Texas, 12 fox squirrels, *Sciurus niger rufiventer*, and 4 gray squirrels, *Sciurus carolinensis carolinensis*, from Angelina, Hardin and Trinity Counties in southeast Texas were examined for helminths. One additional specimen from the Rice Institute campus was examined, and is included in the percentages of parasitic infections mentioned below. No significant differences were noted in the parasitic fauna of squirrels from the several localities represented.

Members of the genus *Sciurus*, as far as can be judged from the literature, are not often heavily parasitized, and the parasites reported vary widely from place to place. In Hall's (1916) monograph on nematodes of rodents, *Trichostrongylus delicatus* was the only nematode reported from squirrels in North America, and it was described as a new species. *Heligmodendrium hassalli* (Price, 1925) is the only helminth so far found to be prevalent in tree squirrels in this country. First reported from Maryland, it was found by Harkema (1936) in over 90% of gray squirrels (*Sciurus carolinensis*) in North Carolina and is here reported from every specimen examined of both gray and fox squirrels from East Texas. It is presumably widely distributed in the southern states. Several kinds of larval tapeworms have been reported from tree squirrels both in this country and in Europe, but *Raillietina* (*Raillietina*) *bakeri* herein described is, so far as I am aware, the only adult tapeworm yet described from *Sciurus* in North America; in Europe *Catenotaenia dendritica*, a member of the family DILEPIDIDAE, is a familiar parasite of

TABLE 1.—*Helminths of tree squirrels in southeast Texas*

	<i>Sciurus niger rufiventer</i> 13 examined		<i>Sciurus carolinensis carolinensis</i> 4 examined	
	No.	%	No.	%
Cestodes				
<i>Raillietina</i> (<i>R.</i>) <i>bakeri</i> ...	8	61	0*	0
<i>Cysticercus tenuicollis</i>	1	8	0	0
Nematodes				
<i>Strongyloides robustus</i> ...	11	85	2*	50
<i>Heligmodendrium hassalli</i> :	13	100	4	100
<i>Microfilaria alpha</i>	1	8	0	0
<i>Microfilaria beta</i>	1	8	0	0

* Two of the gray squirrels and two of the fox squirrels examined were young. All four of these animals were free of all helminths except *Heligmodendrium hassalli*.

Received for publication, May 20, 1941.

squirrels, and a *Hymenolepis* (*H. myoxi sciurina*) has been reported from these animals in Europe and Asia. No trematodes, so far as I am aware, have hitherto been found in tree squirrels, nor were any found in the present investigation.

More detailed data on the helminths found, with descriptions of the new species, follow.

Raillietina (*Raillietina*) *bakeri* n. sp.

(Figs. 1-3)

Delicate worms reaching length of about 60 mm with maximum width of about 1.2 mm. Scolex 250 to 375 μ in diameter, broadest at middle, and without sharp demarcation from neck. Rostellum surrounded by double crown of about 66 hooks appearing as single row, alternate hooks being set only about 2 μ out of line; hooks about 20 to 22 μ long and about 13 μ across the hammer-shaped head; diameter

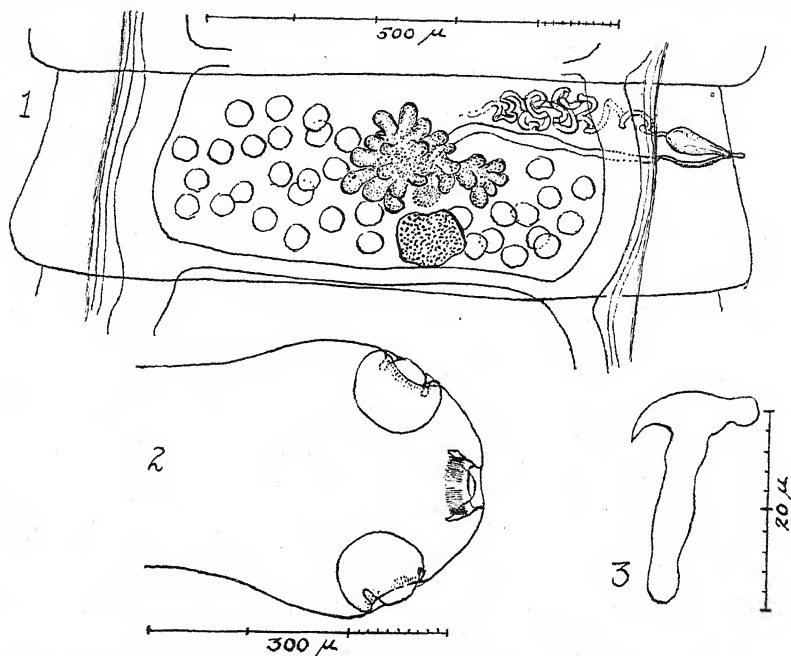


FIG. 1. *Raillietina* (*R.*) *bakeri*, n. sp. Mature segment.

FIG. 2. Same, scolex.

FIG. 3. Same, rostellar hook, greatly enlarged.

across top of crown of hooks 65 to 70 μ . Suckers 80 to 100 μ in diameter with plug of tissue filling most of cup, and provided with very minute spines, 4 to 5 μ long, in at least two rows; most specimens, however, devoid of spines after preparation. Beginning of segmentation about 3 to 5 mm behind anterior end. Neck variable in diameter, according to state of contraction; in well-relaxed specimens usually about 220 to 250 μ , but only 130 μ in one specimen.

Mature segments 680 to 850 μ broad and 175 to 300 μ long; segments showing beginning of uterus usually somewhat narrower but longer, about 550 to 700 μ broad by 400 μ long; segments with developing egg capsules about 800 μ to 1 mm broad and 400 to 450 μ long; undetached segments with egg balls about 1.1 to 1.2 mm

broad, and nearly square. Detached ripe segments usually single or in groups of 2 or 3, from 1.75 to 2.25 mm long and 800 μ to 1 mm broad.

Ventral excretory vessels very broad and conspicuous, up to 60 μ in diameter; transverse vessels slightly narrower; dorsal vessels inconspicuous, seldom seen in older segments. Longitudinal nerve close to dorsal vessel. Genital pores unilateral, situated in anterior third of segment. Cirrus pouch 90 to 95 μ long and about 35 μ wide, not reaching excretory vessels; cirrus unarmed. Vas deferens very much convoluted, occupying a considerable space in anterior poral corner of the segment. Testes 30 to 40 in number, about 18 to 24 of them occupying space on aporal side of segment between female glands and excretory vessel, and 11 to 16 of them on poral side behind genital ducts; in young segments one or two testes may be immediately behind female genital glands, thus connecting the two fields; in older specimens one or two may be dorsal to genital glands. Testes about 28 to 38 μ in diameter, often oval or slightly irregular in shape. Vagina posterior to vas deferens, with muscular deep-staining wall as far as excretory vessels, then becoming widened with thin walls, and serving as seminal receptacle. Ovary situated slightly towards poral side of segment, tending towards division into two main lobes, aporal one the larger, but made up of numerous small finger-like lobes. Yolk gland about 90 to 95 μ in diameter, behind ovary, slightly towards poral side of segment. Segments with developing egg balls detach and finish their development free from strobila, resembling oval seeds. Egg balls remain between excretory vessels, but in fully ripe segments walls of segment close down on egg balls like a capsule, and no traces of excretory vessels or genital ducts can be seen. Egg balls about 80 to 90 in number, round or oval, from 140 to 195 μ in diameter, and containing from 6 to 9, occasionally 10, ova.

Host: *Sciurus niger rufiventer*.

Habitat: Small intestine.

Locality: Southeast Texas.

Type specimen: U. S. Nat. Mus. Helm. Coll. No. 44916. *Paratypes* (4 slides) No. 44917.

Only three species of *Raillietina* have hitherto been recorded from North American rodents, namely *R. (Fuhrmanetta) salmoni* from rabbits, *R. (Paroniella) retractilis* from rabbits, and an undescribed species from a gopher. This is the first species of the subgenus *Raillietina* from North American rodents, and the first adult tapeworm of any kind to be reported from North American tree squirrels. In the small number of testes, small cirrus pouch, and small number of uterine capsules *R. bakeri* more nearly resembles *R. loechesalavezi* Dollfus 1939-1940, described from a specimen from an 18-year-old child in Cuba, than any other form described from mammals. From this it differs, however, in its smaller size, the smaller size of the cirrus pouch, the bilobed uterus, and the failure of the testes to extend beyond the excretory vessels.

Cysticercus tenuicollis

A single small *Taenia* cyst was found attached to the liver of a fox squirrel. The head and neck, evaginated from the cyst but with the scolex invaginated into the neck, measured about 1.85 mm in length, while the cyst measured about 2.2 mm in length. The number, size and shape of the hooks, as well as the general form, indicate that this is a small, probably young, *Cysticercus tenuicollis*, the larva of *Taenia hydatigena*.

This species has previously been reported from squirrels. *Cysticercus fasciolaris* and an unknown cysticercoid with a scolex provided with a double crown of small hooks about 15 μ long (see Schwartz, 1928) have also been reported from tree squirrels in North America.

Strongyloides robustus n. sp.

Parasitic female. Length of egg-bearing worms varying from 4.5 to 6.8 mm, but 6 mm or more in 10 of 13 specimens measured and falling under 5.5 mm in only one case; average length 6.1 mm. Maximum diameter 60 to 75 μ except the 4.5 mm worm, which measured 40 μ . Diameter at base of esophagus 50 to 65 μ except in two worms, in which it was 36 and 40 μ respectively. Cuticle very finely striated. Esophagus 860 μ to 1.26 mm long, averaging 1.14 mm, and varying from 17.7 to 19% of the length of the body. Anus 70 to 80 μ from caudal tip except in the 4.5 mm worm, in which it is 60 μ distant. Vulva 2 to 2.88 mm from posterior end (except 1.6 mm in the 4.5 mm worm), the post-vulvar length representing 35% to 43% of entire body length. Anterior uterus reflexed 325 to 440 μ behind posterior end of esophagus, and posterior uterus 100 to 260 μ anterior to anus. Reflexed loops usually twisted, but to various degrees. Number of eggs in the combined uteri from 3 to 20, averaging 9. Eggs 55 to 60 μ by 27 to 30 μ .

Hosts: *Sciurus niger rufiventer* (type) and *S. carolinensis carolinensis*.

Habitat: Small intestine.

Locality: Southeast Texas.

Type specimen: U. S. Nat. Mus. Helm. Coll., No. 44911.

This is the largest species of *Strongyloides* thus far described except *S. westeri* of horses. It resembles *S. papillosus* in most respects but is larger, the majority exceeding the maximum size reported for *papillosus*. The esophagus is both relatively and absolutely longer, its length in every instance exceeding the maximum length in *papillosus*, and averaging about half again as long as the average for that species. The minimum diameter of the body is equal to the maximum for *papillosus* in all but a single undersized specimen of 13 measured. The vulva in *papillosus* is from 1.6 to 2 mm from the posterior end whereas in *robustus* it is from 2 to 2.88 mm distant, again excepting one undersized specimen. The ovaries are less twisted than in *papillosus*. The egg measurements of *papillosus* are given as 40 to 60 μ by 20 to 25 μ , whereas those of *robustus* are 55–60 μ by 27–30 μ . It differs from *S. agoutii* Griffiths, 1940, in greater size, relatively greater thickness, relatively shorter esophagus, greater distance of vulva from posterior end, larger eggs (46–58 μ by 23–29 μ in *agoutii*). It differs markedly from *ratti* (1) in size; (2) in having an esophagus which, though always longer, is shorter relative to the body length; and (3) in size and shape of the eggs.

This worm was found in moderate numbers in all of the adult fox and gray squirrels examined, but was absent in two specimens of each species which were not full grown.

Heligmodendrium hassalli

This heligmosome was first described by Price (1929) under the name *Heligmostrongylus hassalli* from a gray squirrel, *Sciurus caro-*

linensis, in Maryland, and was subsequently reported by Harkema (1936) from over 90% of specimens of the same host in North Carolina. Dikmans (1931) transferred this species to the genus *Longistriata*, but Travassos (1937) placed it in a new genus, *Heligmodendrium*, on the basis of the interrupted cuticular striations.

Morphological characters and measurements of the Texas worms correspond closely with Price's description of the worms obtained in Maryland, except that the Texas females reach a length of 9 to 12 mm as compared to 8.4 to 8.6 given by Price. In Price's paper the width of the spicules at the base is given as 16 μ , whereas according to my measurements they are only 5 to 6 μ . Examination of Maryland specimens kindly supplied by Dr. Price showed that "16 μ " was evidently a typographical error for 6 μ .

H. hassalli was found, usually in moderate numbers, in every fox squirrel and gray squirrel examined from East Texas, and was the only helminth present in young, half-grown specimens.

Microfilaria alpha n. sp.

Slender unsheathed microfilariae 207 to 237 μ in length and about 3.5 μ in diameter. Head end bluntly rounded, tail drawn out into a fine point. Specimens dried in blood smears lie nearly straight, with only one or two bends in body. Anterior end free of nuclei for distance of about 3 to 4 μ , but nuclei extend almost to tip of tail. Nerve ring 46 to 54 μ (average 50 μ) from anterior end; excretory pore 12 to 17 μ beyond nerve ring. Anal cell 36 to 40 μ from posterior end (average 38 μ). Approximate percentages of body length as follows: nerve ring, 23%, excretory pore, 29%, last genital cell, 70%, anal cell, 82%.

About 150 to 170 μ from anterior end, and 25 to 30 μ anterior to anal cell, a large and conspicuous break in nuclear column representing third "G" cell. About 8 to 12 μ in front of this a somewhat less conspicuous break, probably representing second "G" cell; first "G" cell represented by inconspicuous break, not always seen, about 5 to 10 μ farther anterior. Abundant in blood smears of one specimen of *Sciurus niger rufiventer* from Hardin County.

Host: *Sciurus niger rufiventer*.

Habitat: Blood.

Locality: Hardin County, Texas.

Type specimens: U. S. Nat. Mus. Helm. Coll., No. 44912. *Paratype*, No. 44913.

Microfilaria beta n. sp.

Slender unsheathed microfilariae 125 to 150 μ in length with maximum diameter of about 3.5 μ . Specimens dried in blood smears lie curled in rather kinky manner. Head end bluntly rounded and devoid of nuclei for about 4 to 5 μ . Nerve ring situated about 33 to 40 μ from anterior end (average 36.5 μ). Excretory pore not recognizable. Inconspicuous break in column of nuclei about 75 to 80 μ from anterior end, followed by two others about 7 or 8 μ apart, of which last is largest and most conspicuous. Anal cell 35 to 45 μ from tip of tail. Percentage distances from anterior end average about as follows: nerve ring, 28%, last (largest) genital cell, 69%, anal cell, 85%. This microfilaria was found in very small numbers in blood smears from one specimen of *Sciurus niger rufiventer* from Houston, Texas.

Host: *Sciurus niger rufiventer*.

Habitat: Blood.

Locality: Houston, Texas.

Type specimens: U. S. Nat. Mus. Helm. Coll., No. 44914. *Paratype*, No. 44915.

Very careful search of the carcasses of these two squirrels showing microfilariae in their blood failed to reveal the adults. The body cavities, mesenteries, subcutaneous tissue, inter-muscular fasciae, sinuses, eye sockets and other parts, after prolonged examination, were digested with artificial gastric juice, but without revealing any trace of the adult worms. Hall (1916) reports two species of *Filaria* as having been reported from tree squirrels: *F. pistillaris* Molin, 1858 from Brazil, and *F. linstowi* Hall, 1916 from Siam. Since then *Litomosoides carinii* has also been reported from squirrels, by Travassos in Brazil. The first two species, found in subcutaneous tissue, are very inadequately described and their microfilariae are unknown; *Litomosoides carinii*, abundant in Texas cotton rats (*Sigmodon hispidus*) inhabits the pleural cavities and has sheathed microfilariae. It is probable that both the microfilariae described above represent hitherto undescribed species.

SUMMARY

Thirteen fox squirrels and four gray squirrels from East Texas harbored the following helminths: *Raillietina* (*R.*) *bakeri*, n. sp., in a majority of the adult animals; *Cysticercus tenuicollis*, one specimen; *Strongyloides robustus*, n. sp., in all adult animals; *Heligmodendrium hassalli*, in all animals, even those not full grown; and two kinds of microfilariae, each occurring once.

BIBLIOGRAPHY

- DIKMANS, G. 1935 New nematodes of the genus *Longistriata* in rodents. J. Wash. Acad. Sc. 25: 72-81.
- GRIFFITHS, H. J. 1940 Studies on *Strongyloides agoutii* sp. nov. from the agouti (*Dasyprocta agouti*). Canad. J. Res. 18: 173-190.
- HALL, M. C. 1916 Nematode parasites of mammals of the orders Rodentia, Lagomorpha, and Hyracoidea. Proc. U. S. Nat. Mus. 50: 1-258.
- HARKEMA, R. 1936 The parasites of some North Carolina rodents. Ecol. Monogr. 6: 151-232.
- PRICE, E. W. 1928 Two new nematode worms from rodents. Proc. U. S. Nat. Mus. 74: Art. 4, 5 pp.
- SCHWARTZ, B. 1928 The occurrence of larval tapeworms in the liver, lungs, spleen, kidneys, omentum and heart of the squirrel (*Sciurus carolinensis*). Proc. Helm. Soc. Wash., 118th Meeting, J. Parasitol. 15: 67.
- TRAVASSOS, L. 1937 Revisão da família Trichostrongylidae Leiper, 1912. Monogr. Inst. Oswaldo Cruz pp. 1-512.

HYMENOLEPIS MASTIGOPRAEDITA, A NEW CESTODE
FROM A PINTAIL DUCK*

SEIGUL J. POLK

A pintail duck, *Anas acuta tzitsihoa* Vieillot, collected near Stillwater, Oklahoma, during the fall of 1940, was found to be heavily infected with tapeworms among which were several specimens of an apparently new species herein described as *Hymenolepis mastigopraedita*. The worms were fixed in hot Bouin's fluid and stained in borax carmine. All studies and measurements were made on 11 mounted specimens, 7 of which remain intact and are herewith designated as cotypes.

Hymenolepis mastigopraedita n. sp.

(Figs. 1-4)

Description: Strobila 57-68 mm long, 1.5 mm in maximum width, and about 250 μ thick posteriorly. Longest example with 183 proglottids. No scoleces were found—in most of the specimens, however, the strobila terminates anteriorly in a knot-like body comprising several immature proglottids and regarded as a pseudo-scolex formed apparently by a back-and-forth folding of the front end of the chain followed by adhesion of juxtaposed surfaces, the segments involved being individually recognizable and not noticeably peculiar in form. Youngest available proglottids, 140 μ long, 54 μ wide anteriorly, and 98 μ wide posteriorly. Mature segments somewhat trapezoidal being about 350 μ long, 700 μ wide anteriorly, and 840 μ wide posteriorly. Most fully developed segments, only partially gravid, about 450 μ long and 1.5 mm in maximum width. First-formed proglottid, still present on most of the worms, of diminutive size, rounded posteriorly, and sterile; contains common pore of ventral excretory canals.

Genital apertures unilateral, dextromarginal, and slightly preequatorial. Genital atria bell-shaped, narrow, transversely elongate, and provided each with a prominent, spheroidal, thick-walled, finely spinous sacculus accessorius about 168 μ in diameter in mature segments and located dorsosubanteriad to distal portion of cirrus pouch. Gonaducts dorsal to poral longitudinal urinary canals. Two pairs of longitudinal excretory vessels—ventral ones broad, slightly undulant, and united posteriorly in first-formed segment; dorsal ones very narrow and more sinuous; no transverse commissures observed.

All measurements recorded below for reproductive structures represent averages based upon findings in mature segments.

Ovary comprises two very unequal, irregularly lobulated, and widely separated wings connected by an elongate, narrow, transverse isthmus; greatest diameter of wings—poral 182 μ , aporal 350 μ . Vitellarium 112 μ in diameter, slightly lobed, centrally located between and behind wings of ovary. Female genital pore immediately ventral to male pore. Distal part of vagina an exceedingly tenuous tube, hair-like in appearance; coursing inward from the atrium it makes a single left-hand spiral turn around distal portion of cirrus pouch, passing successively along posterior, dorsal, and anterior surfaces of the latter—the curvature of the spiral sometimes smooth and direct, sometimes complicated by minute sinuosities. Seminal receptacle smoothly

Received for publication, June 17, 1941.

* Contribution No. 94 from the Zoological Laboratory, Oklahoma Agricultural and Mechanical College; prepared under the direction of R. Chester Hughes.

The writer is gratefully indebted to Professor Hans H. Andersen, of the same college, for assistance in translating the Danish text of the paper by Krabbe (1869).

ovoidal, transversely elongate; some $200\ \mu$ long in mature proglottids it becomes very much larger in older ones; submedially located in anterior part of segment ventral to cirrus pouch. Mehlis' gland, oviduct, vitelline duct, and portion of vagina proximal to receptacle—all not observed. In older proglottids, the uterus, partially gravid, nearly fills the segment; it is transversely elongate, irregularly provided with outpouchings, and extends laterally above and beyond excretory canals. No fully gravid segments nor fully developed oncospheres were encountered.

Testes three in number, $308\text{--}320\ \mu$ in diameter, one poral and two aporal; in rather uniform arrangement—two of them being posteriorly located on opposite sides of the median line with the third anterosinistrad from them, all on a level dorsal to the excretory canals and ovary. Vasa efferentia not observed. Cirrus pouch, very prominent and nearly straight in mature segments, $770 \times 90\ \mu$, extends obliquely across body often to protrude into next preceding segment and often to underlap the aporal excretory canals; variously curved and coiled in younger stages. External seminal vesicle prominent, somewhat variable in position but mostly dorsal to cirrus pouch, about $420 \times 112\ \mu$. Internal seminal vesicle, $80\ \mu$ in diameter, largely fills aporal half of cirrus pouch. The two vesicles connected by a narrow sinuous passage that runs through distal end of pouch. Vas deferens between internal vesicle and cirrus describes an elongate, transversely arranged, figure-of-eight loop. Cirrus proper, $280 \times 20\ \mu$, with thick but seemingly nonmuscular wall; apparently only slightly protrusible—only in occasional segments does it project beyond the margin; armed with very fine hair-like spines and provided with a cirrus whip, a very long, slender, hollow filament which arises near the base of the cirrus and extends through its lumen and well beyond its tip—some $300\ \mu$ long, the whip appears to be chitinous and somewhat stiff although the free end is variously looped and coiled about.

Host: *Anas acuta izitsihoo* Vieillot, 1816 = *Dafla a. t.* (V.).

Habitat: Lumen of small intestine.

Locality: Stillwater, Payne County, Oklahoma.

Cotype specimens: Several complete strobilae mounted on separate slides, in U. S. Nat. Mus. Helm. Coll., No. 36807.

According to Hughes (1941) the genus *Hymenolepis* Weinland, 1858, contains some 328 recognized-as-valid species and varieties. The new form was studied in comparison with descriptive data (mostly from Hughes, 1941) on all of the previously known species. So far as the writer has been able to determine only eleven other members (enumerated below) of the genus have been described as having a structure comparable to the cirrus whip. Except as otherwise indicated the original descriptions of these species were used in the present study. Differing from *Hymenolepis mastigopraedita* n. sp. with regard to features severally mentioned:

(1) *H. collaris* (Batsch, 1786) Fuhrmann, 1908, as described by Krabbe (1869) under the name of *Taenia sinuosa* (Zeder, 1800) Rudolphi, 1810, and by Joyeux and Baer (1936), has the testes very considerably smaller, the cirrus proper more protrusible, and the cirrus whip heavier and less protrusible;

(2) *H. columbae* (Zeder, 1800) Railliet and Henry, 1909, as described by Fuhrmann (1906) and Southwell (1930) under the name, *H. sphenoccephala* (Rudolphi, 1810) Fuhrmann, 1906, and by Joyeux and Baer (1936), has a shorter cirrus pouch, characteristic angles in the

course of the vagina, the sacculus accessorius farther posteriad with respect to the cirrus pouch, and the cirrus stylet apparently not protrusible;

(3) *H. cyrtoides* (Mayhew, 1925) Fuhrmann, 1932, is of much smaller size and has an unarmed cirrus and no sacculus accessorius;

(4) *H. flamingo* Skrjabin, 1914, is much smaller and has the cirrus pouch and stylet both much shorter and broader and the genital atrium surrounded by a chitinous ring;

(5) *H. krabbeella* Hughes, 1940, as described by Fuhrmann (1906) and Joyeux and Baer (1936) under the name, *H. fragilis* (Krabbe, 1869) Fuhrmann, 1906, has the testes arranged in a straight line and the cirrus pouch twice as long as the width of the proglottid;

(6) *H. lintoni* López-Neyra, 1932, as described by Linton (1927) under the name, *H. "macracanthos"* (Linstow), apparently has no sacculus accessorius;

(7) *H. longistyllosa* Tseng Shen, 1932, has the testes arranged in a transverse row, a very small cirrus pouch, the cirrus whip many times as long as the width of the proglottid, and no sacculus accessorius;

(8) *H. macracanthos* (von Linstow, 1877) Fuhrmann, 1906, has a 3-lobed ovary, no sacculus accessorius, and the cirrus pouch much longer than the width of the proglottid according to Fuhrmann (1924);

(9) *H. serrata* Fuhrmann, 1906, and the variety,

(10) *H. serrata birmanica* Meggitt, 1924, have the testes arranged in a straight line; and

(11) *H. venusta* (Rosseter, 1897) Railliet and Henry, 1909, has a much shorter cirrus pouch, the internal seminal vesicle folded back and forth in a characteristic series of appressed loops, and the testes disposed in a transverse row according to Rosseter (1898).

The occurrence of the pseudoscolex in the new species would seem to be very unusual if not unique within the genus. In each of the enumerated forms the scolex is known and armed with 8 or 10 rostellar hooks. *H. flamingo* was taken from *Phoenicopterus antiquorum*; *H. columbae*, *H. serrata*, and *H. s. birmanica* are parasitic in columbiform birds; all of the other worms nominately mentioned above were described from anseriform hosts.

BIBLIOGRAPHY

- FUHRMANN, O. 1906 Die *Hymenolepis*-Arten der Vögel. Centr. Bakt. 1. Abt. Orig. 41: 440-452.
———1924 *Hymenolepis macracanthos* (v. Linstow) considerations sur le genre *Hymenolepis*. J. Parasitol. 11: 33-43.
HUGHES, R. C. 1941 A key to the species of tapeworms in *Hymenolepis*. Tr. Am. Micr. Soc. 60: 378-414.
JOYEUX, C. AND BAER, J. G. 1936 Cestodes. Faune de France 30: 1-613.
KRABBE, H. 1869 Bidrag til Kundskab om Fuglenes Baendelorme. K. Dansk. Vidensk. Selsk. Skr., naturv. og math. Afd., ser. 5, 8: 249-363.
LINTON, E. 1927 Notes on cestode parasites of birds. Proc. U. S. Nat. Mus. 70(7): 1-73.

- MAYHEW, R. L. 1925 Studies on the avian species of the cestode family Hymenolepididae. Illinois Biol. Monogr. 10(1): 1-125.
- MEGGITT, F. J. 1924 The tapeworms of the Rangoon pigeon. Parasitology 16: 303-312.
- ROSSETER, T. B. 1898 On the generative organs of *Drepanidotaenia venusta* (*Taenia venusta* Rosseter, 1896). J. Quekett Micr. Club, Ser. 2, 7(42): 10-23.
- SKRJABIN, K. J. 1914 Beitrag Zur Kenntnis einiger Vogelcestoden. Centr. Bakt. 1. Abt. Orig. 75: 59-83.
- SOUTHWELL, T. 1930 Cestoda. In "The Fauna of British India, Including Ceylon and Burma" by Stephenson, London 2: 1-262.
- TSENG SHEN 1932 Etude sur les cestodes d'oiseaux de Chine. Ann. Parasitol. 10: 105-128.

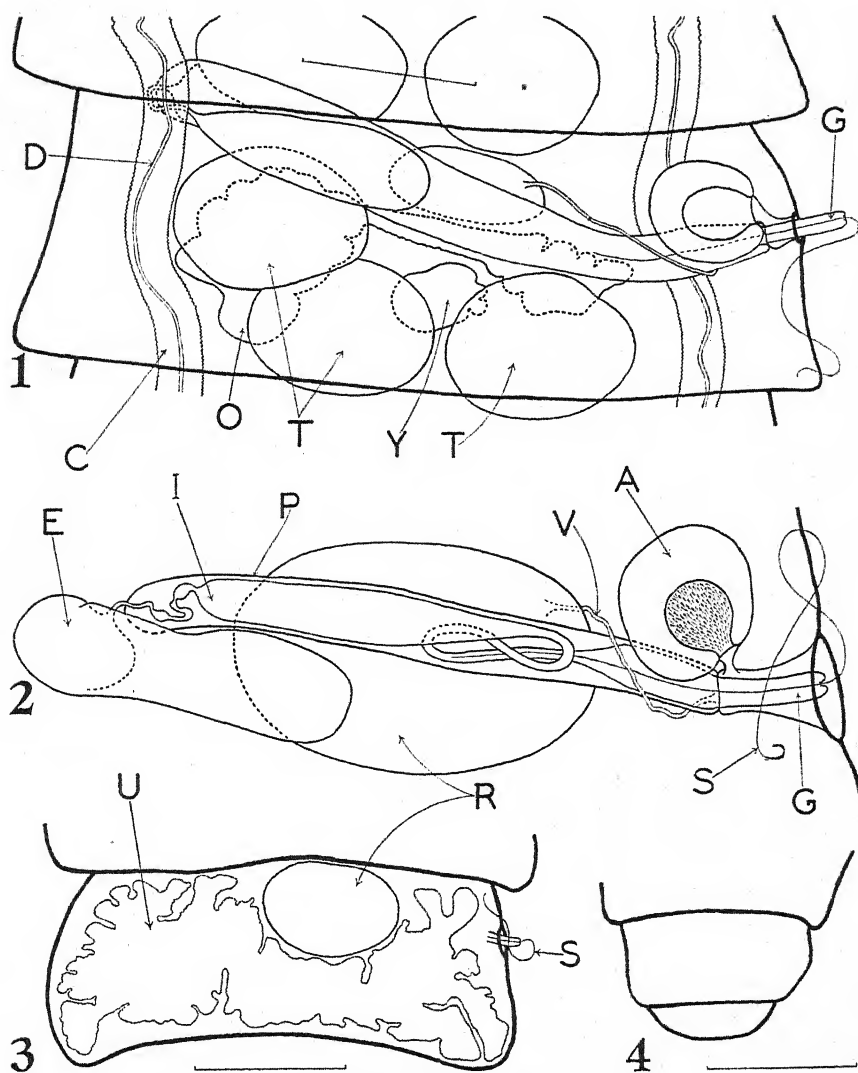
EXPLANATION OF PLATE

All figures concern *Hymenolepis mastigopraedita* n. sp.

ABBREVIATIONS

A—sacculus accessorius	O—ovary
C—ventral longitudinal excretory canal	P—cirrus pouch
D—dorsal longitudinal excretory canal	R—seminal receptacle
E—external seminal vesicle	S—cirrus whip or stylet
G—cirrus	T—testes
I—internal seminal vesicle	U—uterus
	V—vagina
	Y—vitellarium

- FIG. 1. Mature proglottid. Dorsal view. Camera lucida study. Scale, 250 μ .
- FIG. 2. Freehand study of genital tracts in a partially gravid segment. Dorsal view. The spines in the sacculus accessorius are more numerous, more elongate, and more hair-like than the figure suggests.
- FIG. 3. A gravid segment drawn with the aid of a projection apparatus. Scale, 500 μ .
- FIG. 4. Terminal segments of a strobila, drawn with the aid of a projection apparatus. Scale, 1 mm.



Hymenolepis mastigopraedita n. sp.

HAEMOGREGARINA TRIATOMAE N. SP. FROM A SOUTH
AMERICAN LIZARD *TUPINAMBIS TEGUIXIN* TRANS-
MITTED BY THE REDUVIID *TRITOMA*
*RUBROVARIA*¹

J. J. OSIMANI²

Talice in 1929, examining the rectal contents of the blood-sucking reduviid *Triatoma rubrovaria* (Blanchard 1843) coming from the Cerro de Montevideo, noticed "croissant"-like cells which he considered as sporozoites of a haemogregarine. In 1939 we made the same finding in several nymphs and adults of *Triatoma rubrovaria* coming from Maldonado, which also contained *T. cruzi*. If they were, indeed, the final sporogonic forms from a haemogregarine, what was their vertebrate host? The problem, as we see, was reversed from the way it commonly develops with haemogregarines whose species have been described in great number only in the vertebrate. In this case, we knew the intermediate host; the question was to find the definitive host. Talice had suggested that the definitive host might possibly be the small lizard *Teious teyou*, in whose burrows *Triatoma rubrovaria* frequently dwells. The blood examination of six specimens of that lizard, coming from that place, proved negative in 1939. However, later on, in 1940, examining a young lizard which came from Maldonado, we were successful in finding it parasitized with haemogregarines. The lizard specimen was classified by the learned Director of the Natural History Museum of Montevideo, Dr. G. Devincenzi, as *Tupinambis teguixin* L.

In the blood films stained by Pappenheim's method, the parasites showed the following appearance: worm-like shaped cells, slightly curved, measuring $16\ \mu$ in length and $5.5\ \mu$ in width, with rounded and very similar ends. In some examples a kind of fine, long fissure, parallel to the longer axis of the protozoon, which sometimes penetrates very deeply in the body (Figs. 6 and 8) is seen at one end. This does not seem to be an artifact but rather the folding of the parasite within its capsule, a phenomenon which is known in many haemogregarines. There is a prolonged nucleus, smaller than half the length of the haemogregarine; the longer axis parallel to the longer axis of the latter. It stains violet and is placed near the convex side of the parasite, near the boundary between the middle third and the terminal third.

Received for publication, June 19, 1941.

¹ From the Division of Parasitology (Prof. R. V. Talice, Chief) of the Institute of Hygiene of Montevideo (Prof. A. N. Berta, Director).

² I wish to express appreciation to Dr. Clay G. Huff for help received in the preparation of this manuscript for publication.

It is evident from examples 6 and 8, if we admit the explanation given above, that the relations of the position and length of the nucleus would be different. The cytoplasm is of a light blue color without metachromatic granules. It appears in nearly all the examples surrounded by a colorless halo, doubtless a real capsule which impedes sometimes the penetration of the stain, as it happens in Figs. 2 and 3, in which the nucleus is not visible.³ The longer axis of the parasite is parallel to the longer axis of the red cell, which is generally increased, showing very often a hyper- or hypochromatic cytoplasm. The nucleus of the red cell, altered sometimes in its tinctorial reactions, is pushed back to the ends or to the sides of the cell.

On the membrane of the parasite is sometimes noticed a small red spot (Fig. 4) analogous to one noted by Reichenow in *H. stepanowi* and which he considers to be a residue of volutine.

The characteristics previously described belong, no doubt, to gametocytes. We looked for the schizogonic forms in the sections of several internal organs stained by eosin-haematoxylin. After a long search which we had already foreseen owing to the very small number of blood parasite cells, we found in the liver a division cyst. It is an elliptical cyst (Fig. 10), measuring 20 μ in length and 14 μ in width, with a double wall, the internal one being fine and smooth and separated from the external by a fine colorless halo, the external wall being thick (it measures 2 μ on its thickest side), of irregular surface and staining violet by haematoxylin. However, it is possible that what looks like an external wall may really be calcium deposited around the cyst. To determine this point, it would be necessary to stain by special methods. The cyst seems to be confined within an epithelial cell whose nucleus is greatly misshapen.

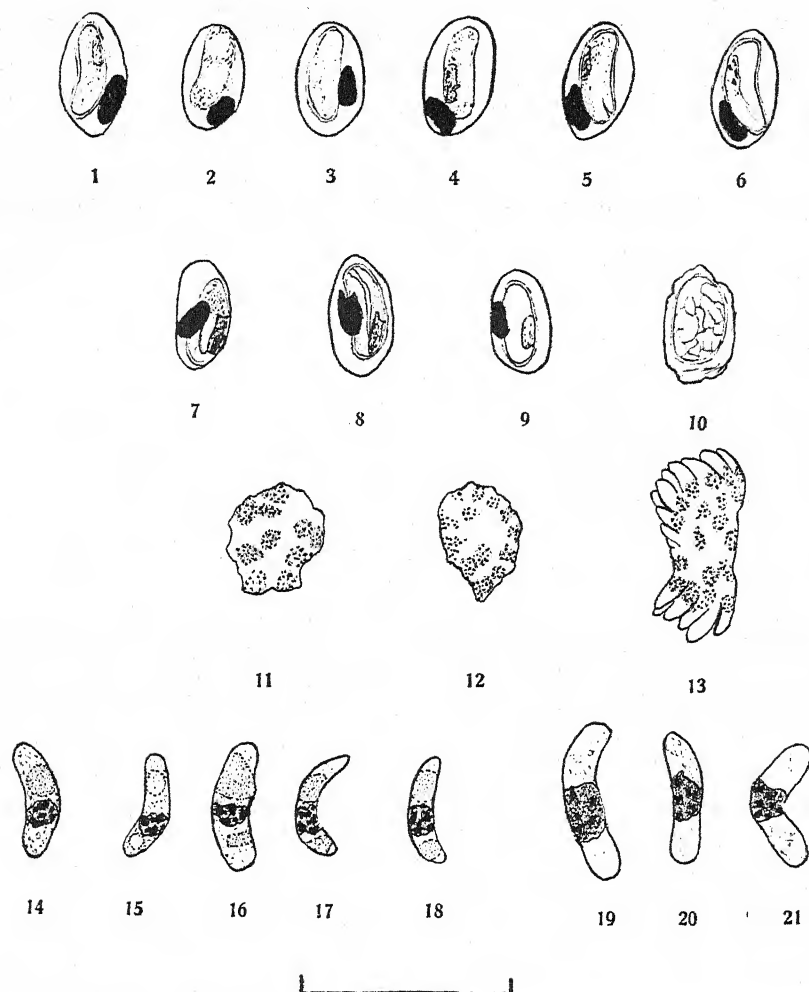
Coming back to the starting point, is this haemogregarine, found in *Tupinambis teguixin* (and over whose classification we do not ponder for the time being), identical with that found spontaneously in the "vinchuca"⁴ *T. rubrovaria*?

Availing ourselves of "vinchucas" from the colony kept in our Institute for making xenodiagnosis of Chagas' disease, we fed nymphs and larvae of these laboratory-bred *T. rubrovaria* on infected lizards. After the blood meal these insects were kept at a temperature of 30° C for two months and then examined.

In many of them we limited the examination to the rectal contents, for it was there that we had found the spontaneous infections. On others we made total dissections of the abdominal contents, studying the midgut, the Malpighian tubes, the genital organs, and the general cavity. Of 30

³ However, the hypothesis that they might be empty capsules, abandoned by the parasite, cannot be dismissed.

⁴ Common name for the blood-sucking Reduviidae in Uruguay and Argentina.



FIGS. 1-21. Reference line, 20 microns.

FIGS. 1-9. Intracorpuseular forms from the blood of the lizard, *Tupinambis teguixin*.

FIG. 10. Schizogonic cyst in the liver of *Tupinambis teguixin*.

FIGS. 11-13. Sporogonic forms in the general cavity of *Triatoma rubrovaria*; in 13 is already seen the segmentation of the oöcyst.

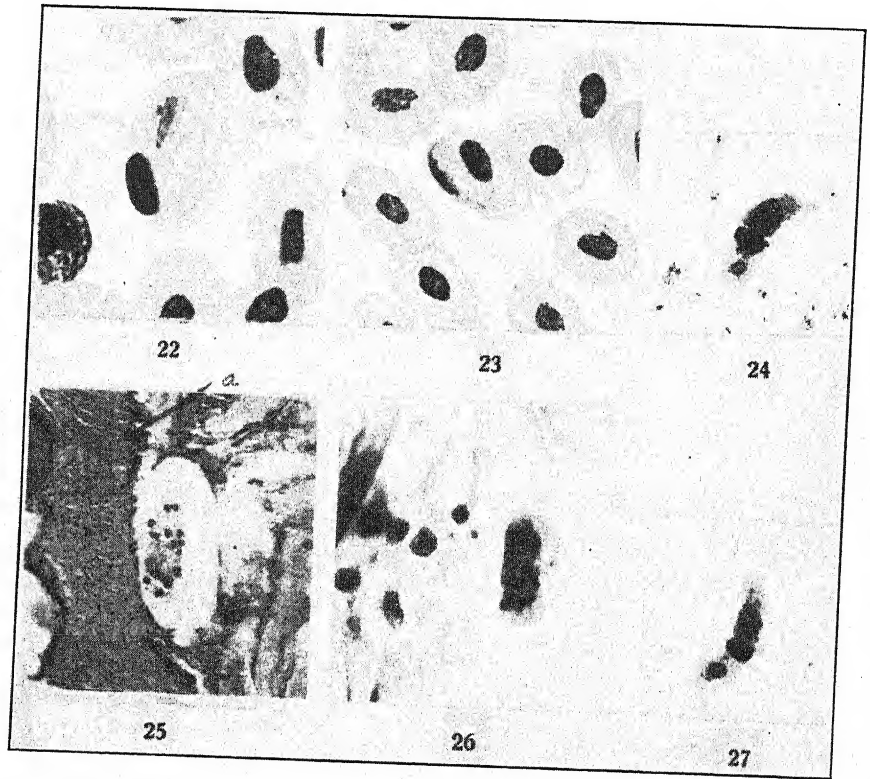
FIGS. 14-18. Sporozoites from the rectum of *Triatoma rubrovaria*, spontaneous infection.

FIGS. 19-21. Sporozoites from the rectum of *Triatoma rubrovaria*, experimental infection.

examples fed upon *Tupinambis teguixin*, only one was positive.⁵ The percentage of infection would be only 3.3% in this experiment, but the

⁵ In more than 500 "vinchucas" used in the Laboratory for making xenodiagnosis of Chagas' disease, these parasites have never been found.

limited blood infection of the vertebrate must be taken into consideration. First we shall describe the cells found in the "vinchucas" with spontaneous infections, and afterwards those examined in the experimental infection of *T. teguixin*.



FIGS. 22-23. Photomicrograph of red cells from *Tupinambis teguixin* parasitized by *Haemogregarina triatomae*. $\times 1000$.

FIG. 24. Photomicrograph of sporozoite from the rectum of *Triatoma rubrovaria*. $\times 650$.

FIGS. 25-26. Photomicrograph of the formation of the oöcyst in the general cavity of *Triatoma rubrovaria*; a. cuticle of the insect. $\times 500$.

FIG. 27. Photomicrograph of the sporozoite from the rectum of *Triatoma rubrovaria*. $\times 650$.

SPONTANEOUS INFECTION OF THE INTERMEDIATE HOST

Examination of the rectal contents showed two kinds of organisms: free sporozoites and cysts. The cysts, elliptical in shape, contain a great number of sporozoites which are clearly seen through the transparency of their smooth and fine wall. The cysts measure from $60\ \mu$ to $80\ \mu$ in length and $42\ \mu$ to $48\ \mu$ in breadth. They contain from 20 to 40 sporozoites. The unstained sporozoites look like crescents, with a sharper end, a

rounded and clearly visible central or sub-central nucleus and a granulated cytoplasm. Stained by the Pappenheim's method (Figs. 14 to 18) the nucleus appears misshapen; the reticulated cytoplasm shows condensations on both sides of the nucleus. Its length varies between 18μ and 30μ , its breadth between 4μ and 7μ . The examination of the Malpighian tubes and the genital organs was negative, nor could we find the enormous cysts of the intestinal cell wall which Talice mentions in his report. The morphological characters of these sporozoites are more or less analogous to those mentioned by Talice.

EXPERIMENTAL INFECTION OF THE INTERMEDIATE HOST

In the experimental material we found only cysts. They are elliptical in shape, with a smooth and fine wall through which the sporozoites can be seen (about 30). The sporozoites are left free by the flattening and breaking of the cysts. These, stained by the Pappenheim's method (Figs. 19 to 21), have an appearance similar to those in the spontaneous infections, but with a larger nucleus and a cytoplasm which is different at the two ends, and without condensations. They measure from 20μ to 25μ in length and 5μ to 6μ in breadth.

On examining sections of insects in paraffin, we looked for early stages of the sexual phase. In this way we noticed that during the growth of the protoplasmic mass, originated perhaps by the conjugation of the gametes, its nucleus begins to divide (Fig. 11), the protoplasmic mass grows longer and the nucleus migrates to the periphery (Fig. 12). In Fig. 13 the sporozoites have begun to develop in the ends. Later on, all the protoplasmic mass changes into sporozoites which line themselves up parallel to the longer axis of the cyst. In this way the cyst is formed.

We cannot state in which of the insect organs such development takes place. We exclude, as explained above, the Malpighian tubes and the genital organs. We have not found parasites in the intestinal wall. The forms which we described (Figs. 11 to 13) were found in the general cavity. Relying only upon the examination of the insect, everything suggests (similar host, localization, and morphology) that the haemogregarine found in spontaneous infection of *T. rubrovaria* by Talice (1929) and me in 1939, and that found in the experimental infection are a single species. The slight morphological dissimilarities between the sporozoites might be explained by the fact that we were dealing with matured sporozoites in the spontaneous infection while those we saw in the experimental infection would not yet be mature. Thus the haemogregarine observed in the lizard, *T. teguixin*, undergoes its sporogonic cycle in the "vinchuca," *T. rubrovaria*, terminating in the formation of sporozoites.

The infectious nature of these sporozoites, however, will not be established until the inoculation of lizards with parasitized bugs has been

attained. Since uninfected lizards were not available at the time we did our experiments, the cycle remains uncompleted.

IDENTIFICATION

"The numerous species of haemogregarines described are nearly all distinguished from one another only by their different hosts, and only in a few cases is our knowledge about them enlarged with the description of their phases of development" (Reichenow, 1920). Certainly the characters which are used for identifying the haemogregarines (except the host species) are generally morphological differences, sometimes rather undefined, which may correspond to different stages or forms of the parasite (micro- or macrogametocyte, young or adult gametocyte). So it frequently happens that it is difficult to affirm whether or not a given form belongs to a species previously described. By now four species of haemogregarines have been described from *Tupinambis teguixin*. These are *H. tupinambisi* Laveran and Salimbeni, 1909; *H. carini* (Laveran) 1909; *H. missoni* Carini, 1909; and *H. iguanae* Ducceschi, 1914. Plimmer (1912) limits himself to mentioning a haemogregarine discovered by him in *T. teguixin* without giving any description.

In the inclosed table the characters of these four species may be compared with those of the species discovered by us. The differences among *Haemogregarina carini*, *missoni*, and *iguanae* are very clear. In the adult form of *H. tupinambisi* there are more analogies, but these analogies do not suffice for identifying them. Writers speak about the great volume increase of the parasitized red cells, which attain 25 μ to 28 μ . In our case the red cells never reach 20 μ . *H. tupinambisi* shows red compact granules, while we did not notice any. The nucleus of *H. tupinambisi* is central and oval in shape; that of our haemogregarine is never in the center, but between the center and one of the ends; besides, it is genuinely prolonged.

We firmly believe then that we have before us a new species, a schizogonic cyst of which we have found in the liver of the lizard and several sporogonic forms of which we have found in the blood sucking reduviid, *T. rubrovaria*. We propose for it the name *Haemogregarina triatomae*.

Haemogregarina triatomae n. sp.

Hosts: Vertebrate: South American lizard, *Tupinambis teguixin*. Invertebrate: *Triatoma rubrovaria*.

Morphology: Erythrocytic forms (gametocytes) worm-like, slightly curved, 16 μ long, 5.5 μ wide, ends very similar. A long fissure parallel to longer axis appears in some, apparently a folding of parasite on itself. Nucleus prolonged, smaller than half the parasite and parallel to length of parasite, violet (with Pappenheim's stain). Cytoplasm light blue, no metachromatic granules, surrounded by colorless halo or capsule. Red cell generally increased in length by parasite which lies lengthwise in it. Cytoplasm of host cell may be hypo- or hyperchromatic. Host nucleus sometimes altered in staining reaction and pushed aside or to end of cell. Small red spot on membrane of parasite.

TABLE 1.—*Haemogregarines of the lizard Tupinambis teguixin*

	<i>H. carini</i>	<i>H. missoni</i>	<i>H. tupinambisi</i>	<i>H. igneae</i>	<i>H. triatomae</i>
Protoplasmic alteration of the red cell	None	None	Great volume increase, reaching 25 μ to 28 μ ; misshapen form, light lilac color	None	Generally an increase of volume; hypo- or hyperchromatic cytoplasm
Nuclear alteration of the red cell	Sometimes peripheral	Normal position	Misshapen, hypertrophied, leaning towards one side or towards the end; sometimes divided in two parts	Scarce	Rejected towards one side or towards the end; sometimes modified in its tinctorial reactions
Shape of the parasite	Vermicular	Kidney-like	Cylindrical	Kidney-like	Vermicular, slightly curved, some specimens seem folded on themselves
Size	15–18 μ \times 3–4 μ	8–9 μ \times 2.5–3 μ	14–16 μ \times 5–6 μ	13.5–15.5 μ \times 3.5–5 μ	16 μ \times 5.5 μ
Capsule			Surrounded by colorless halo		Surrounded by colorless halo
Granules	Fine and red, with deep staining		Thick and red		None
Position of the nucleus	In the center	Central	Central, with its longest axis perpendicular to that of the parasite		At junction of middle third and terminal third; close to the convex side of the parasite; longest axis parallel to that of the parasite
Shape of the nucleus	Prolonged, with very irregular outlines	Oval or round	Oval	Prolonged of irregular outlines	Prolonged
Size of the nucleus	7–12 μ ; $\frac{1}{2}$ or $\frac{2}{3}$ of length of parasite			Much larger than one-half of the parasite's length	Much less than one-half of the parasite's length
Other forms observed in the blood	Forms with queerly rounded ends (feminae?), with light cytoplasm and oval nucleus	None	Young forms of 10 μ \times 2.5 μ ; one end thick and round, the other fine and a little curved. The cytoplasm with fine granules and the central nucleus prolonged with its axis parallel to that of the parasite. Without capsule. Red cell slightly altered with nucleus not disjunct	Young forms (queer) smaller, of 6 μ and 4 μ	None
Schizogony	Not found	Not found	Not found	Not found	Division cyst in the liver
Sporogony	Not found	Not found	Not found	Not found	In <i>Triatoma rubrovaria</i>

Schizogonic stages: In liver of *T. teguixin*, elliptical, 20 μ long, 14 μ wide, with double wall, inner one fine and smooth, external one thick (maximum 2 μ), irregular, violet (by haematoxylin). Cyst apparently in epithelial cell with misshapen nucleus.

Cysts: In *Triatoma rubrovaria*, elliptical, 60–80 μ long, 42–48 μ wide, with large number of sporozoites (20 to 40).

Sporozoites: Crescent-shaped, 18–30 μ long, 4–7 μ wide, one end sharper, rounded central or subcentral nucleus, granulated cytoplasm; when stained they show condensations on both sides of nucleus. In sporogony nucleus divides while protoplasmic mass is enlarging, then migrates to periphery. Sporozoites form and line up parallel to long axis of cyst.

Type locality: Maldonado.

Type host: *Tupinambis teguixin*.

SUMMARY

A new species, *Haemogregarina triatomae*, in the lizard *T. teguixin* L. is described. The infection was scanty; we succeeded in finding, besides the blood stages, a division cyst in the liver. Of 30 "vinchucas" from laboratory-bred *Triatoma rubrovaria* fed upon the infected lizard, one showed different sporogonic forms, including sporozoites, in its tissues and in its rectal contents. These sporozoites are similar to those found by Talice (1929) and by the author (1939) in the rectal contents of "vinchucas" of the same species spontaneously infected.

BIBLIOGRAPHY

- BRUMPT, E. 1938 Formes évolutives d'*Haemogregarina mauritanica* chez la tique *Hyalomma syriacum*. Ann. Parasitol. 16: 350–361.
- CARINI, A. 1909 Sobre duas hemogregarinas do *Tupinambis teguixin*. Rev. Soc. Scient. São Paulo 4: 1–3.
- DEVINCENZI, G. 1925 Fauna erpetológica del Uruguay. An. Mus. Hist. Nat. (Montevideo) 2: 1–64.
- DUCCESCHI, V. 1914 Note di parassitologia comparata del sangue. Ann. Ig. Sperim. 24: 269–273.
- DI PRIMO, R. 1925 Contribução para o Estudo das Hemogregarinas brasileiras. Tesis prof. Edit. Leuzinger, Rio Janeiro.
- FOLEY, H. AND CATANEI, A. 1925 Hémogregarines de sauriens d'Algérie. Arch. Inst. Pasteur Alger. 3: 344–351.
- 1927 Hémogregarines de trois couleuvres et d'un lézard d'Algérie. Arch. Inst. Pasteur Alger. 5: 109–119.
- LAVERAN, A. 1909 Au sujet des hemogregarines de *Tupinambis teguixin*. Compt. Rend. Soc. Biol. 16: 9–10.
- MARQUES DA CUNHA, A. AND MUNIZ, J. 1908 Sobre o cyclo endogeno da *Haemogregarina leptodactyli* Lésage, 1908. Mem. Inst. Oswaldo Cruz 20: 307–315.
- PLIMMER, H. G. 1912 On certain blood parasites. J. Roy. Micr. Soc. p. 133–150.
- REICHENOW, E. 1920 Die Hamogregarinen. In "Handbuch der Pathogenen Protozoen," by von Prowazek und Nöller. Ambrosius Barth, Leipzig.
- TALICE, R. V. 1929 Parasitisme de *Triatoma rubrovaria* par un sporozoaire. Ann. Parasitol. 7: 257–261.
- WENYON, C. M. 1926 Protozoology. Baillière, Tindall and Cox, London.

AGE RESISTANCE OF MICE TO *TRYPANOSOMA CRUZI*

JAMES T. CULBERTSON AND WALTER R. KESSLER

Department of Bacteriology, College of Physicians and Surgeons,
Columbia University, New York

The trypanosome of Chagas' disease, *Trypanosoma cruzi*, is known to cause more severe infections in young persons than in adults, and when the organism is transferred to laboratory rats, mice, and rabbits, young animals likewise usually suffer more intense infections than older individuals (1-4). The guinea pig, on the contrary, is about equally susceptible to the parasite at all ages (3).

Although mouse infections with *Trypanosoma cruzi* have been studied by several investigators, the precise age at which mice attain their comparative resistance is still uncertain since the specific ages of the animals used in the studies reported hitherto has not generally been known. In the present work, an attempt has been made to fix this age for mice, by studying carefully the relative susceptibility of mice of different known age groups. The infections were carried out both by intraperitoneal inoculation of the parasites and by their administration per os.

MATERIALS

The strain of *Trypanosoma cruzi* was obtained originally from the London School of Hygiene and Tropical Medicine in 1936, and has since been kept in this laboratory in the rat or in culture. For administration in the present work, cultures were prepared by adding 0.1 cc of infected rat heart's blood to 5 cc of nutrient broth. The cultures were used after from four to six weeks, when many organisms had developed.

The mice were of the Swiss strain, propagated in the Department of Animal Care of Columbia University.

THE COURSE OF INFECTION IN MICE GIVEN *T. cruzi* INTRAPERITONEALLY

Mice ranging in age from newly-born to 100-day old animals were infected intraperitoneally with doses of *T. cruzi* culture in proportion to body weight, 0.1 cc of culture being given per 10 gm weight. At intervals of 1 or 2 days thereafter, the tail blood of the mice was examined for the presence of trypanosomes, the total number in 50 microscope fields ($\times 430$) being recorded. The data on representative mice from a total of 77 animals are given in Table 1.

Young mice suffered distinctly heavier infections with the parasite than older mice. Animals below 25 days old when infected invariably succumbed (47 animals), whereas those above this age usually survived.

Received for publication, July 4, 1941.

TABLE 1.—Resistance of representative mice of different age groups to *Trypanosoma cruzi* administered intraperitoneally

Mouse No.	Age when infected (days)	Total number of parasites in 50 fields ($\times 430$) on designated day after infection															
		9	10	12	14	16	18	20	22	24	26	28	30	32	34	36	
1	Newborn	0	2	5	12	236	d										
2		1	5	3	36	165	865	d									
3	5	0	0	1	1	6	150	d									
4		0	0	0	1	8	12	d									
5	8	0	0	3	1	3	16	d									
6		0	0	5	9	6	23	d									
7	10	0	2	16	140	d											
8		0	1	6	1	70	d										
9	14	0	0	0	0	1	0	1	5	18	10	d					
10		0	0	0	0	0	0	0	10	35	34	d					
11	20	0	0	1	1	7	3	1	18	d							
12		0	0	0	3	0	6	28	d								
13	25	0	0	0	1	21	3	17	15	86	36	56	65	2	2	0	
14		0	0	2	2	6	15	24	d								
15	33	0	0	0	0	0	0	0	1	0	2	3	2	1	0	0	
16		0	0	0	0	0	0	2	0	5	0	6	2	2	0	0	
17	46	0	0	0	0	0	0	1	0	0	1	0	1	1	0	0	
18		0	0	0	1	0	0	1	0	2	7	7	8	5	4	0	
19	74	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	
20		0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	
21	100	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	
22		0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	

d = dead.

The prepatent period of the infection in animals below 25 days of age at infection also was shorter than that of the mice above this age, its length for the two groups being, respectively, 7 to 12 days, and 14 to 26 days.

THE COURSE OF INFECTION IN MICE GIVEN *T. cruzi* BY MOUTH

An amount of the trypanosome culture roughly proportional to the body weight was administered by mouth to a total of 74 mice of different age groups. Great care was exercised to avoid trauma in administering the dose, a droplet of the culture merely being placed in the opened mouth of each animal. Control mice of each age group were infected at the same time intraperitoneally with 0.1 cc of culture per 10 gm body weight.

TABLE 2.—Relative susceptibility of mice of different age groups to infection after per os administration of *Trypanosoma cruzi*

Age group (days)	Number in group		Number positive		Percentage positive	
	By mouth	I-P	By mouth	I-P	By mouth	I-P
Newborn	8	2	8	2	100	100
2	9	9	9	9	100	100
6	7	7	7	7	100	100
9	6	6	6	6	100	100
12	13	13	13	13	50	100
16	11	11	11	11	15	100
20	6	6	6	6	27	100
25	7	7	7	7	0	100
40	4	4	4	4	0	100
85	3	1	0	1	0	100

At intervals of 1 or 2 days thereafter, the tail blood of the animals was examined microscopically ($\times 430$) for trypanosomes. The relative susceptibility of the mice, in terms of the number and percentage infected in each age group, after per os and after intraperitoneal administration of the parasites is shown in Table 2. The relative intensity of infection in representative mice after administration of the parasite by the two methods is shown in Table 3.

TABLE 3.—Comparative intensity of representative infection after per os and after intraperitoneal administration of *Trypanosoma cruzi*

Mouse No.	Age when infected (days)	Route of infection	Number of parasites in 50 fields (×430) on designated days after infection													
			8	10	12	14	16	18	20	22	24	26	28	30	32	
1	Newborn	Per os	0	0	0	0	0	0	1	4	30	34	16	3	0	
2		" "	0	0	0	0	0	0	0	1	1	4	0	0	0	
3		" "	0	1	36	270	d									
4		I-P	1	5	84	310	d									
5	3	Per os	0	0	0	0	0	0	1	1	0	0	1	0	0	
6		" "	0	0	0	0	0	1	0	0	2	1	3	3	0	
7		I-P	0	0	3	7	13	24	d							
8	9	Per os	0	0	0	0	1	4	2	5	1	7	2	0	0	
9		" "	0	0	12	33	3	3	2	5	2	2	27	46	d	
10		I-P	0	2	4	4	d									
11	11	Per os	0	0	3	3	1	3	2	1	2	0	0	0	0	
12		I-P	0	0	8	27	d									
13	15	Per os	0	0	0	0	0	1	0	0	0	0	0	0	0	
14		I-P	0	2	1	0	1	2	8	d						

d = dead.

Mice of groups below 9 days of age when exposed to per os infection were invariably susceptible to *Trypanosoma cruzi*, the parasites being observed later in their tail blood. Among the mice of groups from 9 to 16 days old at exposure, only 50 per cent or less became infected. Animals 20 days old or more when exposed were regularly refractory to infection by organisms given per os. In contrast, all the control animals infected intraperitoneally with the culture showed the parasite later in the blood stream.

The intensity of infection among the control group injected intraperitoneally was distinctly greater than that of animals which received the organisms by mouth. Most of those which developed an infection after the per os administration survived the disease, whereas the intraperitoneally inoculated controls for these animals invariably succumbed. Usually, the prepatent period after the administration of organisms by mouth was longer than that after their intraperitoneal injection.

The results offered from the present study in mice are essentially confirmatory of those previously published from this laboratory upon age resistance of rats to *Trypanosoma cruzi* (4). Very young mice, like the young rats, evidently are unable to check infections with this trypanosome and soon succumb to it, whereas older animals seem to overcome the parasite with little trouble.

The infections in mice with this strain of *Trypanosoma cruzi* appear usually to be of distinctly lower intensity than those in rats. Often, in fact, the infections even in young mice are so poor as to seem inconsequential until a day or so before the host's death, when many organisms come suddenly into the peripheral blood. Generally, even so, the peak intensity of the mouse infections is considerably below that of rats of corresponding age. This difference may result from the fact, previously stated, that the strain of *Trypanosoma cruzi* used has been kept for several years in the albino rat.

The susceptibility of mice to infection by mouth is likewise essentially the same as that of the rat (5). The less intense infections of mice exposed by mouth compared with those injected intraperitoneally is probably explained by the smaller number of parasites which succeed in entering the animals after administration by mouth. Invasion by fewer parasites prolongs the prepatent period and increases the possibility for the host to check the invader. When the prepatent period is short, as it sometimes is after per os administration, however, the infection usually progresses to the fatal end expected after intraperitoneal administration of the parasite.

SUMMARY

Mice below 25 days of age when infected generally succumb to *Trypanosoma cruzi* administered intraperitoneally, whereas mice above this age usually survive the infection. The infections in young animals develop after a shorter prepatent period and attain greater intensity than those in older animals.

Mice below 9 days of age can regularly be infected with *Trypanosoma cruzi* administered by mouth, and those from 9 to 16 days old often prove susceptible. Mice 20 days old or more resist the parasite given orally. Infections resulting from oral administration of *Trypanosoma cruzi* are generally less intense and less frequently fatal than infections following the intraperitoneal administration of the parasite.

BIBLIOGRAPHY

1. REGENDANZ, P. 1930 Der Verlauf der Infektion mit *Schizotrypanum cruzi* (Chagas) bei jungen Ratten und über die Unempfänglichkeit erwachsener Ratten für *Schizotrypanum*. Centr. Bakt. 116: 256.
2. NIIMI, S. 1935 Studies on experimental Chagas disease. Japan. J. Exper. Med. 13: 543.
3. ZUCCARINI, J. A. 1930 Etudes experimentales sur le *Trypanosoma cruzi*. Compt. Rend. Soc. Biol. 105: 113.
4. KOLODNY, M. H. 1939 Studies on age resistance against trypanosome infection. I. The resistance of rats of different ages to infection with *Trypanosoma cruzi*. Am. J. Hyg. 29(C): 13.
5. KOLODNY, M. H. 1939 V. The influence of the age of the rat upon experimental infection with *Trypanosoma cruzi* per os. Am. J. Hyg. 29(C): 155.

NEMATODIRUS TORTUOSUS N. SP. (NEMATODA) FROM
THE RAT, *NEOTOMA*

HAL TUCKER*

Department of Zoology, The University of California at Los Angeles

During the summer of 1940 nematodes of the genus *Nematodirus* Ransom (1907) were obtained from the small intestines of two species of rats of the genus *Neotoma*. The rodents were trapped in the vicinity of the campus of the University of California at Los Angeles. In May five females and one male were taken from a *Neotoma lepida intermedia*; in July two females and 18 males were found in a *N. fuscipes macrotis*. Since morphological studies showed that these worms represented a species as yet undescribed, the name *Nematodirus tortuosus* is proposed for them.

Nematodirus tortuosus n. sp.

(Figs. 1-6)

Diagnosis: Nematodirus.

Male: 16.00-16.80 mm long, maximum diameter 125 μ . Cephalic cuticular dilation 125 μ long by 60 μ wide, with transverse striations. Body with longitudinal cuticular ridges throughout. Cervical papillae absent. Esophagus 470 μ long. Excretory pore 485 μ from anterior end. Bursa outspread, 500 μ wide. Two large lateral, two small dorsal lobes. Rays long, delicate. Externodorsal thin, 155 μ long; dorsal ray bifurcated in distal 2/7, lateral raylet being longer and itself bidigitate. Margin of bursa distal to small, numerous bosses corrugated by fine ridges about 2.5 μ apart running out to and causing a serrated bursal border. Spicules 2.38-2.90 mm long, equal or unequal in length, yellow-brown in color, with transverse striations. Free proximal parts, measuring about 420-440 μ long by 15 μ in diameter; fused distally forming a single structure with a maximum diameter of 22 μ . Finger-like tip contained in a loose membranous bulb. Pre-bursal papillae absent.

Female: 23.50-28.75 mm long, maximum diameter 245 μ at vulva. Cephalic dilation 130 μ long by 65 μ wide, transversely striated. Anterior two-thirds of body with, posterior third without longitudinal cuticular ridges. Esophagus 500 μ long. Excretory pore 525 μ from anterior end. Vulva 7.35 mm from posterior extremity. Vagina a transverse slit about 60 μ long. Ovejectors very long, combined length including sphincters being 1.55-1.60 mm. Moderate diminution in body size behind vulva to 200-250 μ from posterior end where diameter decreases abruptly to truncated tail. Tail 75 μ long with bristle-like process 13 μ long. Ova smooth-shelled, oval, measure 150-178 by 75-88 μ .

Hosts: *Neotoma lepida intermedia* and *Neotoma fuscipes macrotis*.

Location: Small intestine.

Locality: West Los Angeles, California.

Type specimens: U.S.N.M. No. 36717; paratypes No. 36718. Other paratypes in the Department of Zoology, University of California at Los Angeles.

As far as can be determined, the genus *Nematodirus* Ransom (1907) was last revised in 1937 by Travassos. In his opinion the genus included

Received for publication, September 20, 1940.

*The writer is indebted to Dr. G. H. Ball for the privilege of working in his laboratory and for valuable help and guidance.

16 species (although he lists 17). His list is: *Nematodirus filicollis*, *N. spathiger*, *N. weinbergi*, *N. roscidus*, *N. mauritanicus*, *N. neotoma*, *N. molini*, *N. abnormalis*, *N. helvetianus*, *N. dromedarii*, *N. tarandi*, *N. leporis*, *N. mugosaricus*, *N. aspinosus*, *N. oiratianus*, *N. triangularis*, *N. urichi*.

In regard to the relationship of *N. tortuosus* to the other members of the genus, we find it must be classified as a form occupying a position between the *filicollis* and *neotoma* groups (May, 1920). The first of these was characterized by comparatively short spicules and the location of the vulva near the junction of the middle and posterior thirds of the body. The *neotoma* group, conversely, had relatively long spicules; the vulva was set well back. Members of both groups had divergent ovejectors and uteri.

N. tortuosus occupies an intermediate position. The vulva in the *filicollis* group is placed so that the ratio of total body length to the tail-vulva distance is 3:1. *N. neotoma* has the vulva at or slightly in front of the middle of the posterior third of the body, 5 or 6:1. *N. tortuosus* has a ratio of 3.6:1. From this position the uteri diverge, one passing into the anterior, the other into the posterior part of the body.

The ratio of spicule- to body-length likewise illustrates the point. For *N. filicollis* this is approximately 1:12, for *N. neotoma* 1:3. *N. tortuosus* has a ratio of 1:6.

N. tortuosus is most closely related to *N. aspinosus* Schulz, 1929 (= *N. neomexicanus*), and to *N. leporis* Chandler, 1924, both of which have been found in rodents. *N. aspinosus* resembles it in body length and in general bodily proportions such as the distances from the tail to vulva and anus. The spicules are comparable in that they are unequal and connected throughout most of their extent, but they are less than half as long as those of *N. tortuosus*, the ratio of spicule- to body-length being only about 1:16. The female differs in having no process on the tail and in the greater size of the ova (209–215 by 116–123 μ).

N. leporis is a smaller form measuring 8.0–13.0 mm for males and 16.5–20.0 mm for the length of females. The location of the vulva, the size of the ova, the general character of the bursa, are much the same as in *N. tortuosus*. The spicules of *N. leporis* however are relatively much shorter (0.65–1.05 mm) than those of *N. tortuosus*. On the other hand, the transverse striations on their unequal proximal parts and their fusion into a single structure which ends in a finger-like tip makes them resemble those of *N. tortuosus* more than those of any other species.

The relation of *N. tortuosus* to *N. neotoma* is of particular interest because both have been found only in rats of the genus *Neotoma*. *Nematodirus neotoma* Hall, 1916, has been taken from *Neotoma desertorum*, *N. fallax*, *N. baileyi*, and from *N. rupicola*; *Nematodirus tortuosus* from

two different species: *Neotoma lepida intermedia* and *N. fuscipes macrotis*. The morphology of the nematodes likewise definitely sets them apart: *N. neotoma* is a smaller form with males measuring 8.0–10.5, females 18.3–22.2 mm. The spicules are proportionately longer than those of *N. tortuosus*. The ratio of the posterior extremity-anus distance to total body length is 1:170 as compared to 1:350 for *N. tortuosus* and the vulva has a posterior position which serves to differentiate the females of the two species.

BIBLIOGRAPHY

- MAY, H. G. 1920 Observations on the nematode genus *Nematodirus*, with descriptions of new species. Proc. U. S. Nat. Mus. 58: 577–588.
TRAVASSOS, L. 1937 Revisão da família Trichostrongylidae Leiper, 1912. Monogr. Inst. Oswaldo Cruz 1: 278–294.
YORKE, W. AND MAPLESTONE, P. A. 1926 The Nematode Parasites of Vertebrates. P. Blakiston's Son & Co., Philadelphia. Pp. 135–137.

EXPLANATION OF PLATES

Nematodirus tortuosus n. sp.

All figures were drawn with the aid of a camera lucida.

PLATE I

- FIG. 1. Anterior end of male, lateral view.
FIG. 2. Posterior extremity of female, showing spinous process.
FIG. 3. Region of vulva, showing vagina, ovejectors, ova, beginning of uteri.

PLATE II

- FIG. 4. Spicules: a—dorsal view of tips. b—lateral view of tips; c—cross section of fused portion.
FIG. 5. Spicules: a—entire spicules, lateral view; b—proximal unequal portions.
FIG. 6. Bursa, dorsal view.

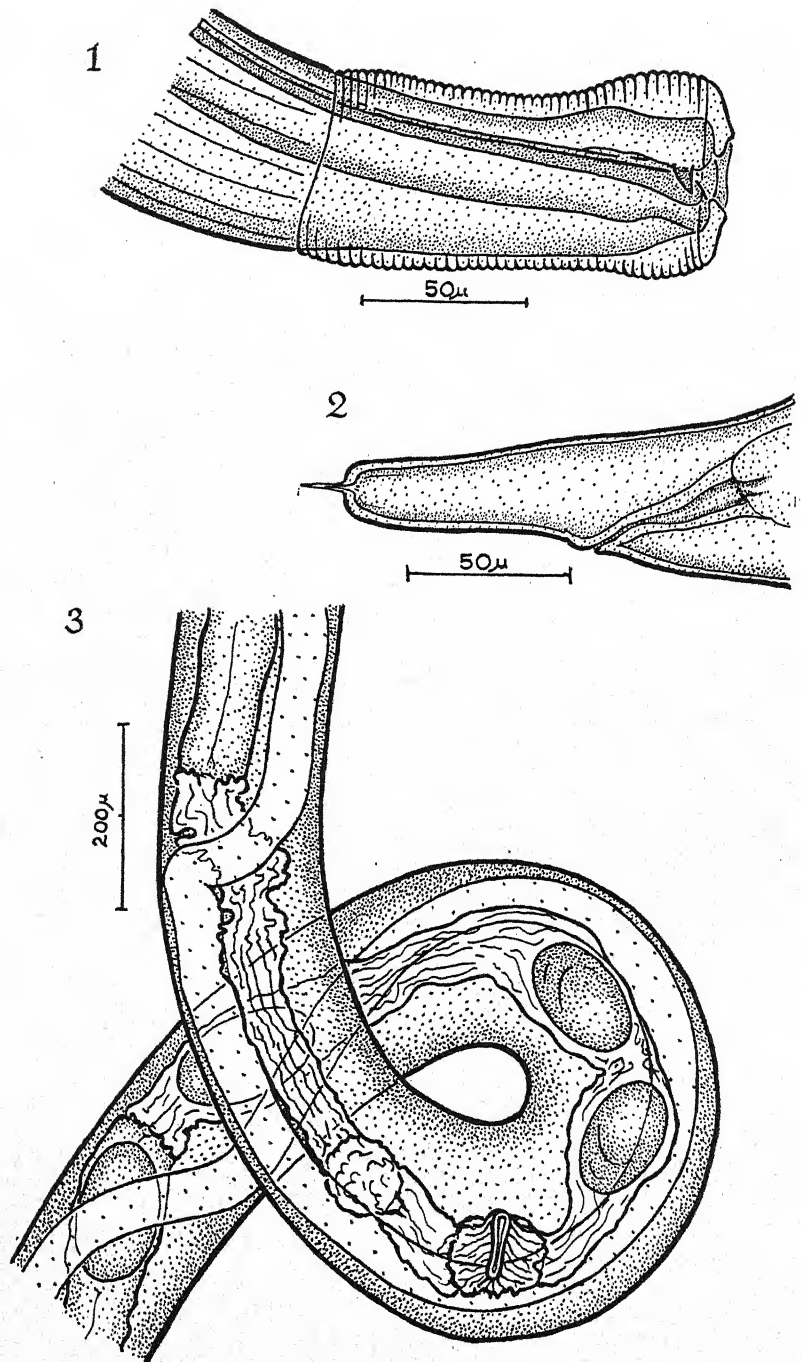


PLATE I

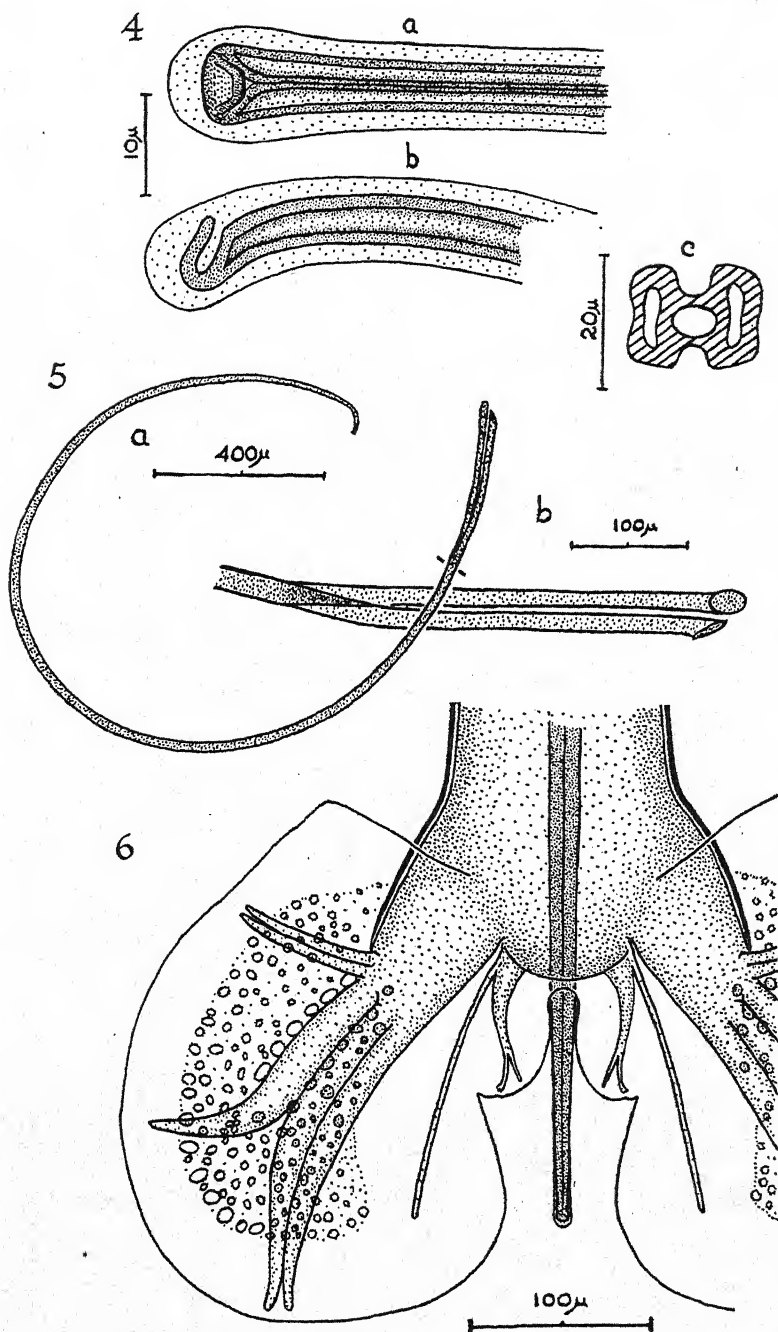


PLATE II

RESEARCH NOTES

NOTE ON THE ASSOCIATION OF THE TICK, *ORNITHODOROS TALAJE* (GUÉRIN-MENEVILLE), WITH BAT INFESTATIONS IN HUMAN DWELLINGS IN BRAZIL

Specimens of a tick were collected by the author in 1939 at Carmo do Rio Claro, Minas Geraes, Brazil, on the ceiling of a farmhouse infested by many bats, and were identified as *O. talaje*. Ticks of the same species from Campo Belo, a neighboring country of the above, had later been sent to Dr. H. Beaurepaire Aragão at the Instituto Oswaldo Cruz by Dr. A. Botelhos, who found them in beds in a house in that area.

In addition, there were frequent reports in Carmo do Rio Claro of a tick found on the ceiling of houses, which was increasing in numbers, and whose bite caused edema and severe itching in persons. The possibility that flies of the families Nycteribiidae and Streblidae, common in this region, were responsible for the bites was discounted when inhabitants of the farmhouses failed to describe them. In addition, these flies are not known to leave their bat hosts.

Large numbers of the bat, *Histiotus velatus* Geoff., were present in 2 farmhouses, and adult and 2 nymphal ticks were captured in a corner where the bats rested during the day.

Specimens of this species were also taken in the bedroom of still another farmhouse. Apparently the ticks fall into the beds from the ceilings which, in old farmhouses of this region of Brazil, are loosely constructed of bamboo. The fact that only larvae or nymphs parasitize the man is probably explained on the basis of a narrower host range in the adult.

Recently (1940) in Viçosa, the author received specimens of this tick collected in a house by the inhabitants who stated that ticks frequently fell from the ceiling. The ticks disappeared when the bats were removed. Bats, therefore, appear to be responsible for the introduction of these pests.

According to Aragão (1936, Mem. Inst. Oswaldo Cruz 31: 759-844), *O. talaje* is common in holes of the rodent, *Kerodon rupestris*. Larvae and nymphs have been collected on *Coelogenes paca* and *Dicotyles tajacu*. That author states that large numbers of that species were found in a cave in Matto Grosso, Brazil, by Dr. Alípio de Miranda Ribeiro who made no reference to bats in the cave, however.

So far as the author is aware, this is the first reference to the occurrence of the tick, *O. talaje*, as parasites of bats inside dwellings. In his opinion, the adult ticks in such places are strictly parasites of bats and removal of the bats also eliminates the ticks.—JOSÉ CANDIDO M. CARVALHO, *Escola Superior de Agricultura e Veterinária, Viçosa, Brazil; and University of Nebraska.*

PARALAEURIS CUCKLERI N. SP. (NEMATODA) FROM THE IGUANA (*CYCLURA CORNUTA*)

In 1938, A. C. Cuckler reported on the nematodes parasitic in a land iguana (*Conolophus subcristatus*) from the Galapagos Islands (Allan Hancock Pacific Expeditions, 2: 137-165. U. of S. Calif. Press) and established a new genus of oxyurid worms—*Paralaeuris* (Syphacinae)—with *P. dorochila* as the type species. The present writer recently had the opportunity of examining a number of parasites collected from a West Indian iguana (*Cyclura cornuta*) which had died in the Zoological Gardens at Washington, D. C. Among the collections were large numbers of both sexes of an oxyurid that not only established the validity of the genus *Paralaeuris*, but added a second species to that genus. The new species is designated as *Paralaeuris cuckleri*, in recognition of the work done by Cuckler on the parasites of the Iguanidae.

Paralaeuris cuckleri n. sp.

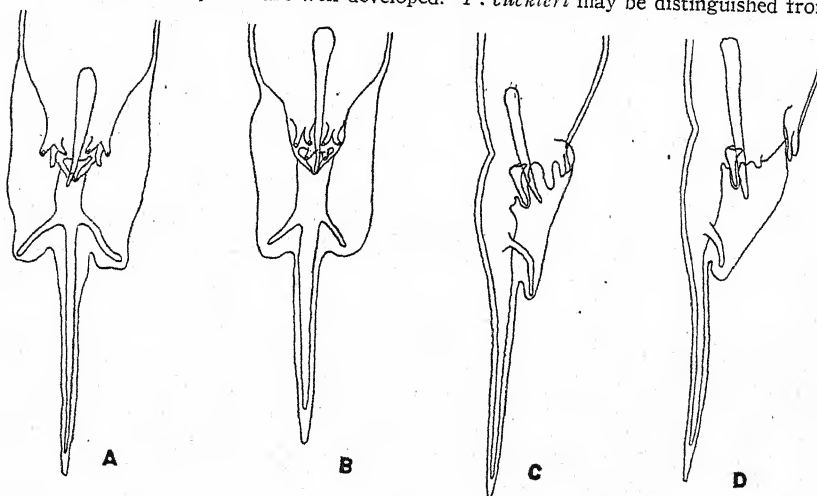
Males: Average of 10 specimens. Length, 2.5 mm; greatest width, 0.125 mm; pharynx length, 0.018 mm; esophagus length, 0.5 mm; bulb length, 0.063 mm; head-nerve ring distance, 0.148 mm; head-excretory pore distance, 0.66 mm; cloaca-tail distance, 0.18 mm; spicule length, 0.081 mm; accessory piece length, 0.018 mm; caudal papillae, two pairs of precloacal pedunculate papillae (almost double papillae), one pair of sessile papillae on the anterior lip of the cloaca, and one pair of pedunculate postcloacal papillae. Pedunculate papillae support distinct caudal alae.

Females: Average of 10 specimens. Length, 3.1 mm; width at vulva, 0.19 mm; pharynx length, 0.018 mm; esophagus length, 0.5 mm; bulb length, 0.063 mm; head-nerve ring distance, 0.15 mm; head-excretory pore distance, 0.75 mm; vulva-tail distance, 1.5 mm; anus-tail distance, 0.72 mm; egg size, 0.027×0.042 mm. Eggs are unsegmented at time of oviposition.

Type host: West Indian Iguana (*Cyclura cornuta*).

Habitat: Posterior intestine of host.

The lips of both sexes are provided with cuticular projections, as in the case of *P. dorochila* Cuckler, and the excretory pore is similarly post-bulbar in position. The vagina and ovejector are well-developed. *P. cuckleri* may be distinguished from



Ventral and lateral views of the tail region of males of *Paralaeuris cuckleri* and *P. dorochila* to show difference in size and arrangement of caudal structures.

- A. *Paralaeuris dorochila*. Ventral view of tail of male.
- B. *P. cuckleri*. Ventral view of tail of male.
- C. *P. dorochila*. Lateral view of tail of male.
- D. *P. cuckleri*. Lateral view of tail of male.

P. dorochila on the bases of (1) the larger size of the male and the corresponding increase in the size of the other male structures, (2) the difference in the structure and arrangement of the caudal papillae of the male, (3) the median position of the female vulvar opening, (4) the smaller size of the eggs, and (5) particularly by the fact that the posterior portion of the esophagus in both sexes is glandular instead of muscular as in *P. dorochila*. All of these points have been checked against cotype material of *P. dorochila*.

The material studied was made available through the courtesy of the Zoological Division of the Bureau of Animal Industry, U. S. Department of Agriculture, and the type material is deposited in their collections.—A. C. WALTON, *Knox College, Galesburg, Illinois*. Contribution No. 77.

A *PARAGORDIUS* LARVA (GORDIACEA) IN A TREMATODE

A single *Paragordius* larva was found in the parenchyma of a trematode taken from the small intestine of the hog molly, *Hymentelium nigricans* collected from Honey Creek near Ann Arbor, Michigan, on May 3, 1941. The trematode was identified as being very closely related to *Plagioporus sinitsini* Mueller, 1934 (Allocreadiidae). One of six trematodes contained, just anterior to the genital pore, a *Paragordius* larva in a very early stage of development. The larva was free in the parenchymatous tissue. No spontaneous movement was visible and there was no indication of a cyst. The larva was very loosely bent into the shape of a letter J, the posterior end being curved. This is the second record of the occurrence of a larva of Gordiacea in a trematode. Cort (1915, J. Parasitol. 1: 198-199) found *Gordius* larvae in the trematode species, *Brachycoelium hospitale* Stafford, 1903, from the intestines of the green newt, *Diemictylus viridescens*. He found eight of sixteen trematodes from several different hosts infected, two containing two larvae and the others one each.—JACOB H. FISCHTHAL, Dept. of Zoology, University of Michigan, Ann Arbor, Mich.

EIMERIA ANTELOCAPRAE, A NEW COCCIDIUM FROM THE AMERICAN ANTELOPE

A young antelope was struck by an automobile some twenty or more miles north of Laramie, Wyoming, February 9, 1941. Authorities of the Wyoming Game and Fish Commission were notified, and the badly hurt animal was killed and brought to the parasitology laboratory at the University of Wyoming stock farm. An examination for parasites was made the following day. A study of the contents of the cecum and large intestine revealed numerous oöcysts.

Two antelope fecal samples collected February 19, 1941, at a spring hole some twenty miles north of Laramie contained the same type of oöcysts as those found in the killed animal. Of seven samples collected in the same area on March 19, three contained the oöcysts, but in these they were not numerous.

To the writer's knowledge the literature includes no record of coccidia from the American antelope. The oöcysts are not like those reported from cattle and sheep (Becker, 1934, "Coccidia and Coccidiosis"; Christensen, 1938, Proc. Helm. Soc. Washington 5: 24; Christensen, 1938, J. Parasitol. 24: 453-467; Christensen and Porter, 1939, Proc. Helm. Soc. Washington 6: 45-48) although sheep and cattle range over the same area. Furthermore, the oöcysts are different from those described from *Orias canna* (Triffitt, 1924), *Capella rupicapra*, *Cervus axis*, *Cervus canadensis*, *Cervus capreolus*, *Cervus elaphus*, *Dama dama*, (Rastegaieff, 1930, Arch. Protistenk. 71: 377-404), and *Gazella subgutturosa* (Yakimoff et al, 1932, Z. Parasitenk. 5: 85-93). These oöcysts are, therefore, believed to represent a new coccidium for which the name *Eimeria antelocaprae* is proposed.

The length and width measurements given in the description of these oöcysts are based on a study of 200 oöcysts: 67 from the killed animal, and 67 from one and 66 from the other of the two fecal samples collected February 19. The differences among the length means and width means of the three groups are not statistically significant. The means and standard deviations for the three groups and for the total 200 oöcysts are as follows:

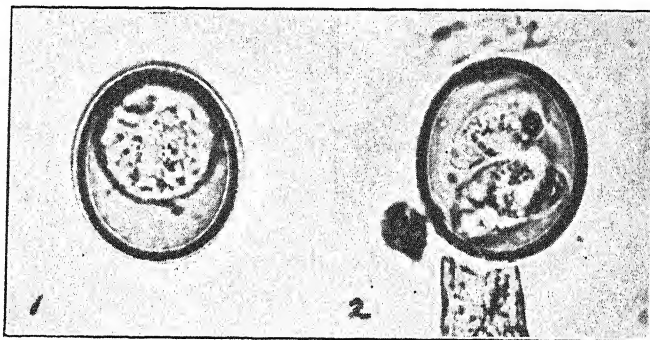
	Group 1	Group 2	Group 3	Total
Number	67	67	66	200
Mean length μ	31.1	31.2	30.2	30.8
St. dev.	2.0	2.5	2.3	2.3
Mean width μ	26.5	26.4	25.3	26.0
St. dev.	1.2	1.7	1.4	1.5

The sporont range in size and mean as given is based on measurements from 25 oöcysts.

Eimeria antelocaprae n. sp.

Figs. 1 and 2

Description of oöcysts: Oöcysts 24.6 to 35.2 μ (average 30.8 μ) long by 21.1 to 29.5 μ (average 26.0 μ) in transdiameter. Shape typically ellipsoidal, from 1.04 to

FIG. 1. Unsporulated oöcyst of *Eimeria antelocaprae* n. sp.FIG. 2. Sporulated oöcyst of *Eimeria antelocaprae* n. sp.

1.34 (average 1.18) times as long as broad. Wall approximately 2.2 μ thick, smooth, transparent, nearly colorless to a faint dull yellow-green in tint with that part of wall just outside oöcyst membrane darker. No micropyle nor thinning of wall at one end apparent. Sporont 16.5 to 19.8 μ (average 17.8 μ) in diameter. Spores approximately 16.5 long by 9.2 μ in transdiameter. Oöcystic residual material present as a few small irregularly shaped particles.

Type host: Antelope (*Antilocapra americana*)

Type locality: Near Laramie, Wyoming.

—HENRY HUIZINGA, Agricultural Experiment Station, University of Wyoming, Laramie.

STUDIES UPON *STRONGYLUS VULGARIS*

VI. TESTS WITH ORGANIC COPPER SALTS

Continuation of in vitro tests upon *Strongylus vulgaris* similar to those reported previously (Whitlock, 1940, J. Parasitol. 26: 45-47; 49-57) has revealed several facts which may be of importance in vivo work with anthelmintics.

The methods used in these tests have been described in detail in the previous papers. Briefly, a population of female *Strongylus vulgaris* was collected from infected ceca at a nearby horse-slaughtering establishment. Test groups were selected at random from this population and exposed to the action of the proper copper salt in warm 0.85% NaCl solution for 100 minutes. Each test contained one control group exposed to 0.85% NaCl alone. At intervals thereafter the worms were tested for the presence of rigor mortis with a special apparatus. Those showing rigidity to resist a perpendicular pressure of 4.4 grams were considered dead.

The chemicals used were CP. Molarity as herein used means the amount of salt per unit of water, not necessarily in solution.

Table 1 gives the results of this series of experiments in which the measurement of efficacy of the concentration of various copper salts used is given by the LT50 (time necessary to kill one-half of the test group). This was estimated as in previous papers by means of graphic analysis of probit mortality. It may be noted that the same concentration of each salt used produces a markedly different effect on different sample populations, but that salts which are constant in solubility produce almost the same relative effect on any given sample population as the standard salt

copper sulphate, a fact noted before in experiments with the cupric halides (Whitlock, op. cit.).

Some of the salts, however, present points of special interest. Copper tartrate, even though relatively insoluble, is effective in destroying *Strongylus vulgaris* in vitro. This is not true of many insoluble inorganic compounds, such as copper arsenite and carbonate. This accounts for copper tartrate's efficacy in destroying nodular worms, as reported by Monnig (1935, Onderstepoort J. Vet. Sc. 5: 419-438). Copper formate is also worthy of notice since it is so much more effective than copper sulphate and since the same concentration of sodium bicarbonate (0.1 per cent) which inactivates 0.01 molar solutions of inorganic copper compounds does not completely immobilize it.

TABLE 1

Experiment number	CuSO ₄	Copper acetate	Copper \odot tartrate	Copper sulpho-carbolate	Copper formate	Copper formate + .1% NaHCO ₃
1	596		645		206	429
2	689	524*	740	454	298	
3	746	714	613	568		
4	562		552		182	
5	1208 ^x				196	460

* Basic copper acetate—has twice as much copper available as ordinary copper acetate.

\odot Solubility is variable.

^x Probably an error.

Note: Each experiment contained one control group whose LT50 was at least three times larger than the largest LT50 in the experiment except in experiment 5.

Three conclusions may be drawn from these data. (1) This paper reemphasizes the existence of a marked secular variation in the reaction of *Strongylus vulgaris* to a lethal agent. Such a marked secular variation must be guarded against in tests in vivo. (2) Organic copper salts, unlike inorganic, need not be water soluble to be effective against a parasite either in vivo or in vitro. (3) Copper formate, because of its markedly lethal action and because of its resistance to inactivation by sodium bicarbonate, should be tried as a substitute for copper sulphate in such diseases as haemonchosis of sheep.—J. H. WHITLOCK, *Kansas Agricultural Experiment Station, Contribution No. 89, Department of Veterinary Medicine.*

INTESTINAL PARASITES IN BOYS OF THE FLORIDA INDUSTRIAL SCHOOL

A survey of 188 white inmates of the Florida Industrial School for Boys (delinquent juveniles), situated in Jackson County, Florida, has been made recently to determine the incidence of helminthic and protozoan parasites. Jackson County in 1937-38 was shown (Leathers, Keller, and McPhaul, 1938, Am. J. Hyg. 87(D): 1-16) to have a hookworm incidence of 44 per cent. Recently gathered unpublished data have shown this county to have an incidence of 60 per cent.

The individuals examined in this survey have come from nearly every county in the state of Florida and represent a high school age group, i.e., 12 to 19 years inclusive.

A single examination of each specimen was performed by each of the three following methods: (1) Within a few hours after passage of the specimen a fecal smear for iron hematoxylin staining was made. (2) All specimens were then examined by both the simplified zinc sulfate centrifugal floatation technic, and (3) the Willis' brine levitation technic. The zinc sulfate in this simplified technic had a specific gravity of 1.2, the same as the salt, and the cheesecloth straining and second washing were omitted.

Intestinal parasites were found in 122 individuals (65 per cent). Nine different species of parasites were recorded. Forty-four (23 per cent) harbored helminths and 107 (57 per cent) were found infected with protozoa.

The simplified zinc sulfate centrifugal floatation technic followed by staining with D'Antoni's iodine was found to be the best general procedure for the recovery of helminth ova and protozoan cysts. Thirty-five (75 per cent) of the helminth infections, 77 (72 per cent) of the protozoan infections, and 94 (77 per cent) of the total parasitic infections were revealed by this method.

TABLE 1

Positives found	Positives found by all three methods		Most efficient method	Per cent positives found by most efficient method
	No.	Per cent		
Hookworm	35	19%	Brine	14%
Whipworm	9	5%	Zinc sulfate	4%
Tapeworm (<i>H. nana</i>) ..	1	0.5%	Zinc sulfate	0.5%
<i>Endamoeba coli</i>	81	43%	Zinc sulfate	34%
<i>E. histolytica</i>	8	4%	Iron H.	3.7%
<i>Endolimax nana</i>	29	15%	Iron H.	10%
<i>Giardia lamblia</i>	18	9%	Zinc sulfate	7%
<i>Iodamoeba bütschlii</i>	6	3%	Iron H.	3%
<i>Chilomastix mesnili</i>	2	2%	Iron H.	2%
Total helminths	44	23%	Zinc sulfate	18%
Total protozoa	107	57%	Zinc sulfate	41%
Total parasite incidence	122	65%	Zinc sulfate	50%

The incidence of helminthic infections was lower than that known for most of the state of Florida and only about one-third that for the county in which the school is located. However, conditions prevailing in this institution would tend to prevent the spread of hookworm infection and this in turn would lower the helminth incidence. Furthermore, hookworm infections in new arrivals at the school are treated. Most cases found in this study were probably residual infections from inefficiently treated cases.

Protozoan infections were three times more numerous in this group than has been noted in the general population in Florida (unpublished studies). It is not known whether the individuals examined obtained their protozoan infections before or after entering the school. Food handlers in this institution were found uninfected.—WILLIAM A. SUMMERS, Senior Parasitologist, Bureau of Laboratories, Florida State Board of Health, Jacksonville, Florida.

A NOTE ON VITAMINS IN RELATION TO ECTOPARASITE RESISTANCE

Searls and Snyder (1935, J. Econom. Entom. 28: 304-310) conducted some experiments on the control of ectoparasites of laboratory rats in 1935. During the course of the work it was noticed that those animals in better health apparently had less lice than those in a poor condition. These incidental observations were repeated many times and it was suggested that perhaps dietary factors had something to do with the condition. Vitamin A was thought of first since there is abundant evidence to show that this factor has a profound effect upon endoparasites and is a physiological prophylactic against infection, in general.

During the years 1936-37 the author conducted experiments at the University of Wisconsin on the relation of vitamin A to resistance of the albino rat to its lice. The results at that time indicated a strong positive correlation between adequate amounts of vitamin A in the diet and resistance of the laboratory rat to the multiplication of the rat louse, *Polyplax spinulosa* Burm.

Searls and Snyder (1939, J. Parasitol. 25: 425-430), basing further experimental work on the preliminary results of Kartman, concluded that "vitamin A was the main factor which regulated the resistance or susceptibility of the rats to the lice."

Further observations upon the problem substantially confirm the preliminary results of Kartman and the work of Searls and Snyder. At this time it is possible

to add actual louse counts taken post mortem which give a quantitative value to the gross observations of louse infestation on the live rats. Rats maintained at a level of avitaminosis-A showed an average infestation of 1451.4 lice per rat; rats given the curative treatment for avitaminosis-A showed an average infestation of 28.2 lice per rat; the control rats showed an average infestation of 12.5 lice per rat. These data are taken only from those rats which lived for 25 days or more after the onset of avitaminosis-A. It was also concluded that neither sex nor litter had any significant effect upon immunity to lice in the rat host.

Notwithstanding the positive evidence cited above, the statement of Searls and Snyder attributing to vitamin A the main limiting role in the ability of the rat to resist the rat louse may be too sweeping, and the problem may not be quite that simple and clear-cut. Gyorgy (1938, *Proc. Soc. Exper. Biol. and Med.* 38: 383-385) showed that pediculosis was apparent in about 20 per cent of rats kept on a riboflavin-free diet for from eight to ten weeks. The lice were almost completely eliminated from the host by the therapeutic feeding of riboflavin. Asa C. Chandler (1941, Farrar and Rinehart, N. Y.) in his recent book on nutrition, "The Eater's Digest," after pointing out that members of the vitamin B₂ complex may be linked with sulphur metabolism, suggests that this may be the way in which riboflavin influences pediculosis.

In any event, it seems clear at this time, that we cannot state that any particular vitamin plays the single or even the "main" limiting role in determining the resistance of rats to their lice. The known work on this problem to date has shown simply that two variates (amount of vitamin in the diet and resistance to lice) maintain a fairly definite relation to each other. However, they tell nothing about the cause of the relationship but merely reveal its existence.

The actual data therefore suggest that vitamin A cannot be taken as the "main factor" in the resistance of albino rats to lice. Rather it is one nutritional variate having a significant relation to possible cutaneous or metabolic changes which may cause a true immunity to develop against lice in the rat host.—LEO KARTMAN, *U. S. Department of Agriculture, Bureau of Entomology and Plant Quarantine.*

COMMUNICATIONS

CARLOS FINLAY AND YELLOW FEVER

To the Editorial Committee of the JOURNAL OF PARASITOLOGY:

I am most appreciative of the lengthy and detailed review of my book "Carlos Finlay and Yellow Fever" published in the August 1941 Journal by Dr. F. L. Soper, and I am in complete accord with his statement that:

"It is indeed regrettable that unpleasant discussions as to who should get the credit for the mosquito transmission of yellow fever have occurred; surely there was glory and to spare for all who had any part in contributing to the work which led to the control of the most fearful of tropical scourges."

the last part of which reproduces almost word for word the remarks of Dr. Guiteras at the banquet, presided over by Gen. Wood in Havana on December 22, 1900. As well as with that:

"Surely it will not be difficult to reconcile rival claims."

My father and I have always been the first to recognize to the full the brilliant confirmatory and complementary work of the Reed Commission, permitting myself to reproduce the following words of my father at the above-mentioned banquet:

"Twenty years ago, guided by indications, which I deemed certain, I sallied forth into an arid and unknown field; I discovered therein a stone, rough in appearance. I picked it up and with the assistance of my efficient co-laborer—Dr. Claudio Delgado—polished and examined it carefully, arriving at the conclusion that we had discovered a rough diamond. But nobody would believe us, till twenty years later, when there arrived a commission composed of intelligent men, who in a short time extracted from the rough shell the stone to whose brilliancy none can now be blind."

I must however take exception to some remarks of Dr. Soper somewhat in contradiction with his conciliatory phrases.

In the first place I object to my book being considered a brief at all and much less as a brief for the plaintiff in the case of Finlay vs. Reed et al, and Gorgas. In any case it might be considered as a brief for the defense against persistent and unwarranted non-recognition of the full scope of my father's work. It is moreover misleading, in this regard, to mix the Reed Commission with Gorgas, as my father never had any issue with the latter, whose share was limited to the sanitary practical confirmation of the mosquito theory, independent of the individual share of the parties concerned.

The issue at the stake between my father and the Commission is the non-admission by the latter of the former's alleged "positive cases" of experimental yellow fever, admittedly fewer in proportion and milder in intensity to theirs, based solely on the duration of the "extrinsic incubation," since they never discussed the cases clinically, an argument possibly tenable at a time when the supposed yellow fever parasite was held, like that of malaria, to spend a phase of its existence in the body of the mosquito, but is no longer so after the brilliant work of the South American yellow fever workers (including Dr. Soper) which shows that the virus in the mosquito is infective from the moment of its ingestion and that the length of time required for it to reach its salivary glands and be infective through the bite varies from four days onwards according to the outside temperature.

I deplore with Dr. Soper the introduction of nationalistic arguments into this discussion, a fault to be found with some participants on both sides of the controversy.

The relative merits of the parties concerned are most clearly set forth in the following documents of strictly North American origin:

I. Gorgas' final Report to Gen. Wood (Report of Brig. Gen. Wood—Report of Chief Sanitary Officer, July 12, 1902; reproduced on page 118 of my book):

"In the summer of 1900 the commission of Army and medical officers headed by Walter Reed, U. S. Army, had been sent to Cuba for the investigation and study of yellow fever. Due to the financial assistance given by the Military Governor to this commission they were enabled to experiment on human subjects. They took up the theory advanced by Dr. Carlos J. Finlay of Havana in the year 1881 that the *stegomyia* mosquito was the sole means of transmission of yellow fever. Dr. Finlay had maintained his theory for some twenty years and had done considerable experimental work in this direction.

The commission, through elaborate and careful experiments proved this to be correct. . . . I myself had seen the work and was convinced that the mosquito could convey yellow fever but I was hardly prepared to believe that it was the only way or even the ordinary way of conveying the disease."

II. Memorial Tablet presented to the City of Havana by the City of New Orleans on December 3, 1941:

Mayoralty of the City of
New Orleans, Louisiana, U.S.A.

To His Honor, the Mayor of the
City of Havana, Cuba.

Greetings:

The people of New Orleans, recognizing their unpayable debt to *Carlos Finlay*, the immortal discoverer of the transmission and propagation of yellow fever by the mosquito; and,

Remembering that it was by the adoption of Carlos Finlay's principles of anti-mosquito sanitation, so decisively confirmed by the heroic labors of the United States Army Yellow Fever Commission under the direction of Walter Reed, and irrefutably demonstrated by the marvelous efficiency of the anti-mosquito campaign conducted by Colonel William C. Gorgas in Havana in 1899-1900, that Havana and Cuba were definitely and permanently rid of the yellow fever pestilence; and,

Since New Orleans, profiting by the example of Havana by adopting the same anti-mosquito tactics and strategy, achieved a definite and decisive victory over yellow fever in 1905, which delivered not only New Orleans but the whole North American continent from the terrors of the Yellow Fever peril, and again,

In view of the unparalleled era of salubrity, prosperity and civic improvement that has followed in the wake of that epochal victory of sanitary science over yellow fever in our city in 1905; and,

Furthermore, in view of the happy and constantly growing commercial, cultural and good neighborly relations between New Orleans and Havana, that has been made possible primarily through Finlay's discovery, it is hereby

Resolved and ordered by the City Council of New Orleans that a copy of this letter be transmitted in suitable form for permanent preservation, to the Mayor of the City of Havana as a testimonial of gratitude and appreciation contributed by the City of New Orleans to the public celebration of the one hundred and eighth anniversary (108th) of the birth of Carlos Finlay which is held in Havana on December 3rd, 1941, and that our honored and beloved fellow citizen, Dr. Rudolph Matas, Commander of the Cuban Order of Carlos J. Finlay, be commissioned to make this presentation in behalf of the City of New Orleans.

The Mayor of the City
of New Orleans.

Yours very sincerely,

C. E. Finlay, M.D.

Vedado, Habana.

CONSERVATION OF SCHOLARLY JOURNALS

To the Editorial Committee of the JOURNAL OF PARASITOLOGY:

We are enclosing a brief statement of one of the aims of the Committee on Aid to Libraries in War Areas.

The American Library Association created this last year the Committee on Aid to Libraries in War Areas, headed by John R. Russell, the Librarian of the University of Rochester. The Committee is faced with numerous serious problems and hopes that American scholars and scientists will be of considerable aid in the solution of one of these problems.

One of the most difficult tasks in library reconstruction after the first World War was that of completing foreign institutional sets of American scholarly, scientific, and technical periodicals. The attempt to avoid a duplication of that situation is now the concern of the Committee.

Many sets of journals will be broken by the financial inability of the institutions to renew subscriptions. As far as possible they will be completed from a stock of periodicals being purchased by the Committee. Many more will have been broken through mail difficulties and loss of shipments, while still other sets will have disappeared in the destruction of libraries. The size of the eventual demand is impossible to estimate, but requests received by the Committee already give evidence that it will be enormous.

With an imminent paper shortage attempts are being made to collect old periodicals for pulp. Fearing this possible reduction in the already limited supply of scholarly and scientific journals, the Committee hopes to enlist the cooperation of subscribers to this journal in preventing the sacrifice of this type of material to the pulp demand. It is scarcely necessary to mention the appreciation of foreign institutions and scholars for this activity.

Questions concerning the project or concerning the value of particular periodicals to the project should be directed to Wayne M. Hartwell, Executive Assistant to the Committee on Aid to Libraries in War Areas, Rush Rhees Library, University of Rochester, Rochester, New York.

The Committee will be grateful for your cooperation in this attempt to notify individual scholars of its hopes and plans.

Sincerely,

WAYNE M. HARTWELL,
Executive Assistant

The Journal of Parasitology

Volume 28

JUNE, 1942

Number 3

ROBERT WILLIAM HEGNER

1880-1942

By the death of Robert Hegner on March 11, 1942, parasitology in the United States has lost another of its important leaders. For almost a quarter of a century he had carried on extensive researches on the parasitic protozoa and had been the director of an active group of students and investigators in this field. He was one of the founders of the American Society of Parasitologists, having been a member of the original committee that drew up its constitution and called the first meeting in Washington in 1925. When the Society took over the JOURNAL OF PARASITOLOGY in 1932 he became the representative of protozoology on the first editorial committee. He was vice-president of the Society in 1928 and its president in 1936. His relations with foreign workers in parasitology have also been unusually close not only from the exchange of publications but also from personal contacts during his extensive travels. To his numerous students he was a close friend and advisor, and his charming personality and rare sense of humor made him a favorite with his colleagues. Parasitologists will long remember the delightfully informal way in which he presided at the meetings of the Society at Atlantic City in 1936.* Those of us who were with him during the last months of his life will never forget the brave fight that he made, when in spite of increasing pain and weakness he refused to give up and continued his lectures and the direction of his department until a few weeks before his death.

Dr. Hegner was born in Decorah, Iowa, on February 15, 1880. He was graduated from Lyons' Institute in Chicago and then continued his education in the University of Chicago where he received the A.B. degree in 1903 and the M.S. degree in 1904. During this period he acted as assistant in the Department of Zoology, spent one summer on a scientific expedition in Mexico, and several summers at the Marine Biological Laboratory at Woods Hole. Later, he went to the University of Wisconsin where he received the Ph.D. degree in 1908, the title of his dissertation being "The Origin and Early History of the Germ Cells in

* Dr. Hegner's portrait, and presidential address given at Atlantic City, entitled "Parasite Reactions to Host Modifications," were published in this JOURNAL, February, 1937 (23: 1).

Some Chrysomelid Beetles." During the years 1908 to 1917, he was an instructor and then assistant professor of zoology at the University of Michigan. At Michigan, he developed further his interest in the germ cell cycle of insects, publishing a series of about 20 papers on this subject and a book, "The Germ Cell Cycle in Animals," which appeared in 1914. During this period, also, he published his "Introduction to Zoology," 1910, and the first edition of his "College Zoology," 1912, which has continued as a leading text book up to the present time. In fact, the fifth edition of the College Zoology was finished just before his death, and will be published this summer.

During 1917 and 1918, he held a Johnston Scholarship in the Department of Zoology of the Johns Hopkins University. Here he carried out his first researches on the protozoa and published a series of five papers on variation and heredity in the free-living species, *Arcella dentata*. While in Baltimore, he received an offer of a position in the newly organized School of Hygiene and Public Health of the Johns Hopkins University. Seeing clearly the possibilities of the field of parasitology in relation to public health, he joined the staff of this institution in 1918. Here he organized the work in medical zoology and developed research and teaching in protozoology. He served this institution for more than two decades, devoting himself to the study of the parasitic protozoa, but fostering, also, active work in the whole field of parasitology. His own researches covered all phases of his subject, and his publications during this period were very numerous, totaling almost 150 titles. In addition, he stimulated research activity in those around him. Forty students received the doctorate under his direction and a number of other investigators studied in his laboratory. After a few years, his department became one of the most active centers of research in parasitology in the world.

Dr. Hegner was a great traveller, and his researches on the protozoan diseases of man took him frequently into the field. He directed several expeditions in tropical America, spent a year in the Philippine Islands, and in 1938 worked for five months at the Institute of Public Health in Mexico City. In addition, he travelled widely in Europe, the Orient, and South America. He was a member of many scientific societies in this country and abroad, and was on the editorial boards of a number of journals of zoology, parasitology, and tropical medicine.

Dr. Hegner's personal contributions to parasitology were many and varied. He was the author of the following books in this field: (1) Diagnosis of Protozoa and Worms Parasitic in Man (with W. W. Cort), 1921; (2) Outlines of Medical Zoology (with W. W. Cort and F. M. Root), 1921; (3) Human Protozoology (with W. H. Taliaferro), 1924; (4) Host-Parasite Relations between Man and His Intestinal Protozoa, 1927; (5) Problems and Methods of Research in Protozoology (with

J. Andrews), 1930; (6) Parasitology, 2nd ed. (with F. M. Root, D. L. Augustine, and C. G. Huff), 1938; and (7) Big Fleas Have Little Fleas, 1938.

In research, while centering his attention on the human protozoa, he also constantly developed the zoological aspects of the subject. It would take too long to try to analyze in detail his research contributions. A number of his papers dealt with the systematics and morphology of the protozoa of man and other animals. Still others gave the results of surveys of human intestinal protozoa in countries which he visited. His main interest, however, was in the study by experimental methods of the factors influencing the transmission of parasitic protozoa and of the relations of the host to its parasites. In this connection he introduced the term "host-parasite relations," which has been widely used. One line of research on host-parasite relations which he started rather early and came back to from time to time was the relation of the diet of the host to its infection with intestinal protozoa. Of most interest, perhaps, in this connection was the finding that a carnivorous diet in mammals is unfavorable for the intestinal flagellates. Another series of his investigations was concerned with the biology of intestinal protozoa, particularly *Trichomonas* and other flagellates, as studied in vitro and in laboratory animals, particularly the rat and newly hatched chicks. Also, on one of his trips to Panama he had the opportunity of studying experimental anebiasis in monkeys and made significant contributions to our knowledge of the histopathology of this disease.

He had early recognized the importance of experimental studies on malaria and using various species and strains of bird malaria, carried out with the aid of a number of his students studies on all phases of the malarial problem. During the last six years, these studies completely dominated his interest and absorbed the whole research efforts of his department. Of the numerous results that have come out of this program on bird malaria, perhaps, the most important have been those that dealt with the significance of acquired immunity and with the relation of the susceptibility of young red blood cells to the course of the infections. In his last paper, published in September, 1941, he reported the successful treatment of bird malaria with a new drug, which is now being tried on human malaria.

Dr. Hegner's personal accomplishments in research were supplemented by the investigations of his numerous students and the visiting investigators in his laboratories. A large proportion of his students are still engaged in research in protozoology, and some already hold positions of leadership. Thus, his influence will long continue. He lived a very full and rich life, and left behind him an unusually large body of accomplishments.—W. W. CORT, *School of Hygiene and Public Health, Johns Hopkins University*.

LIFE HISTORY STUDIES ON TWO TREMATODES OF THE SUBFAMILY NOTOCOTYLINAE*

E. C. HERBER

Dickinson College, Carlisle, Pa.

During the summer of 1939 several species of notocotylid cercariae were isolated from naturally infected snails collected from the Douglas Lake region, Michigan. Cysts formed from cercariae of two of these species were fed to a wide variety of different animals and adults were obtained in suitable hosts. In 1940, intermediate hosts were infected with eggs obtained from the adults of these two species and the early larval stages were followed in the snail intermediate hosts.

A review of the literature showed accounts of about ten experiments in which cercariae and adults were connected in notocotylid life cycles. In four cases the adults were identified as *Notocotylus attenuatus* Rud. [Joyeux (1922), Mathias (1930), L. and U. Szidat (1933), and Yamaguti (1938)]. The others dealt with forms that were identified as six different species [Harper (1929), L. and U. Szidat (1933), U. Szidat (1935), Luttermoser (1935), and Yamaguti (1938)]. In only one case was the snail intermediate host experimentally infected (Joyeux, 1922). Joyeux, therefore, gave the only description of a notocotylid mother sporocyst. Only in a few cases have two generations of rediae been reported for the larval stages in this group.

The present communication deals with life history studies on two species of notocotylid trematodes parasitic in mammals and birds. The first, *Quinqueserialis quinqueserialis* (Barker and Laughlin, 1911), has been reported previously only in the adult stage. Its intermediate stages are found in *Gyraulus parvus* (Say). The second, *Notocotylus stagnicola* n. sp., is previously undescribed in any stage. Its intermediate stages are found in *Stagnicola emarginata angulata* (Sowerby) and *S. e. canadensis* (Sowerby).

MATERIALS AND METHODS

Natural infections of larval stages were obtained in snails collected from Douglas Lake, Walloon Lake, and Burt Lake, Michigan. Mature cercariae were studied alive and also from permanent whole mounts

Received for publication, June 11, 1941.

* Contribution from the Biological Station of the University of Michigan and the Department of Helminthology of the School of Hygiene and Public Health of the Johns Hopkins University, Baltimore, Maryland. The writer wishes to express his indebtedness to Dr. W. W. Cort, who suggested the need for this investigation and whose suggestions and criticisms throughout the course of the work were invaluable.

stained in Ehrlich's acid hematoxylin. Some cercariae were fixed in hot 10 per cent formalin for measuring. Metacercariae were studied alive and as permanent whole mounts stained in paracarmine. Lettuce-lined stender dishes were used in obtaining cysts for feeding experiments.

Of the experimental final hosts, albino and hooded rats, ducks, and part of the chickens were raised in the laboratory. The rest of the chickens were purchased from nearby farms and the meadow mice were trapped. The adult worms were studied alive or fixed in Gilson's fluid heated to about 65° C. Sections were made from material fixed in Bouin's solution. Freshly shed eggs were fixed by dropping into hot fixing solutions.

Laboratory raised snails, *S. e. angulata*, were used for the infection experiments with *N. stagnicola*. Small specimens of *G. parvus* with a low incidence of infection with *Q. quinquesequalis*, were used for the infection experiments with that species. It was therefore necessary to take special precautions so that natural infections would not be mistaken for experimental infections in these snails. A week after exposure to freshly shed eggs, snails were first examined with a dissecting microscope for evidences of infection.

In connection with the study of ventral glands in adult worms a new method was devised by which a permanent impression of fixed worms could be made on the film side of lantern slides. The unused lantern slide is cut to the size of microscope slides and placed in hypo for 20 minutes. Then the strips are washed in water and allowed to dry until the gelatin is of rubber-like consistency. The ventral side of the worm is flattened on this film for several minutes with the pressure of a lead weight on top of a strip of heavy glass. The worm is shortly peeled off and an impression is left permanently on the film which shows clearly the position and number of ventral glands.

THE LIFE CYCLE OF *Quinquesequalis quinquesequalis* (BARKER
AND LAUGHLIN)

The adult stage of *Q. quinquesequalis* is a parasite of the cecum of muskrats and meadow mice. Eggs deposited with the cecal material gradually pass out with the feces. A group of experimental snails, which was examined at intervals for 26 days after exposure to the eggs, gave information on the course of development in the intermediate host (Table 1).

The Eggs and Infection of Intermediate Hosts.—Adult worms, raised experimentally in the meadow mouse, were placed in stender dishes containing saline. A short time later filamentous eggs were extruded from the genital pore in the form of ropy masses. The shell of the egg is light brown, oval, operculate, and has polar filaments. Twenty eggs fixed in

TABLE 1.—Sequence of stages of *Q. quinquesequalis* in the intermediate host, *Gyraulus parvus*, exposed to eggs on July 27, 1940

Age of infection in days*	Number of stages found	Stage of development	Position in snail
9	6	Immature mother sporocysts	Mantle
10	2	" " "	"
11	32	" " "	"
13	8	Mature mother sporocysts	"
14	2	" " "	"
16	3	Mother rediae	Along intestine
17	2	Mature mother sporocysts	Mantle and along intestine
18	38	Mother rediae	Mantle
18	1	Mature mother sporocyst	Along intestine
19	7	Mother redia	Found loose
20	1	Mother rediae with d. rediae	Along intestine
20	1	Mother sporocyst	Along intestine and in digestive gland
20	40	Mother rediae (estimated)	Mantle
21	4	Daughter rediae	Along intestine and in digestive gland
21	1	Mother sporocyst	In digestive gland
22	47	Rediae (mother and daughter)	Mantle
23	32	Rediae (mother and daughter)	Along intestine and in digestive gland
23	14	Immature cercariae	In digestive gland
24	43	Rediae (mother and daughter)	" " "
24	9	Immature cercariae	Along intestine and in digestive gland
25	88	Rediae (mother and daughter)	In digestive gland
25	21	Immature cercariae	" " "
26	46	Rediae (mother and daughter)	" " "
26	11	Immature cercariae	" " "
		Mature cercariae	" " "

* One snail was examined on each of these days.

hot Gilson's had lengths varying from 0.018 to 0.021 mm and widths from 0.011 to 0.014 mm. The lengths of the polar filaments varied from 0.268 to 0.436 mm. In spite of the opacity of the egg shell which made observations of the contents difficult, they could be seen to contain miracidia.

On July 27, 1940, several lots of small specimens of *G. parvus* were exposed to eggs of *Q. quinquesequalis*. A control series of a dozen snails from the same collection was not exposed to eggs. The following descriptions except for older cercariae and metacercariae were made from this experiment in which 25 out of 28 snails were infected with young larval stages. The other three snails were found to have mature natural infections of this species and unfortunately were not examined any further for young larval infections. Controls had a similar percentage of mature natural infections; the rest were negative. Some laboratory raised *Physa gyrina* were also exposed to eggs of *Q. quinquesequalis*, but examinations two weeks later revealed no evidence of infection.

The Mother Sporocyst (Figs. 1, 2).—The youngest mother sporocysts recognized were found imbedded within the mantle lining as early as 9 days after exposure to infection. The sporocyst at this stage is sac-like, oval or round, and measures between 0.023 and 0.026 mm in diameter. The wall is lined with a layer of epithelial cells. A mass of cells is loosely attached to the inside wall. Ten days after exposure more of these oval sacs were found containing germinal masses of different sizes. In later examinations up to 21 days after infection sporocysts of increasing size

were found in which the rediae could be distinguished (Table 1). Some were firmly imbedded in the mantle; others were loosely attached along the intestine, and a few were found loose in the dish when the snail was opened. The largest sporocyst found measured 0.243 mm in diameter. The number of sporocysts found in a single snail varied, the highest being 32. The number of rediae inside the sporocyst is always 4. No birth pore was observed in the sporocyst wall and apparently the mother rediae are able to break through the wall at any point.

The Mother Redia (Fig. 3).—As early as 14 days after exposure small, almost colorless mother rediae were found free along the intestine; the smallest was 0.125 mm long and 0.053 mm wide. After the mother redia inside the sporocyst has developed a pharynx, a narrow hyaline lumen with dilations extends from the pharynx into the body cavity to form the intestine. This later enlarges to form a large sac-like intestine filled with dark yellowish granules and occupying a good deal of the body cavity of the redia. In a large redia the pharynx measured 0.038 by 0.034 mm. The mouth is terminal and capable of considerable protrusion and contraction. Seven mother rediae containing daughter rediae were found in the digestive gland of a snail 19 days after exposure. One of these rediae had 5 daughter rediae and 17 germ balls. The largest mother redia seen measured 0.70 by 0.20 mm. It was impossible in the experimental snails to differentiate immature mother and daughter rediae free in the tissues.

The Daughter Redia (Fig. 4).—Daughter rediae were observed free in that part of the digestive gland nearest to the stomach 20 days after infection. At the time of the escape from the mother redia a daughter redia measured 0.109 by 0.046 mm. Older daughter rediae are yellowish elongate sacs, narrow at the anterior end and wider towards the posterior end. They are like mother rediae and can only be distinguished from them by the presence of developing cercariae. The smallest daughter redia containing embryos that could be recognized as developing cercariae measured 0.41 by 0.16 mm; the largest 1.31 by 0.23 mm. Most mature daughter rediae contain a few well developed cercariae and about a dozen germ balls in various stages of development. In many cases eye-spots in these cercariae are well developed and are visible through the walls of the rediae.

The Cercaria (Figs. 5-8).—Cercariae, almost mature, emerge from the birth pore of the rediae and migrate into the digestive gland where a short period of maturation occurs. Maturing cercariae, 23 days old, exhibited little motion and it was not until 3 days later that actively swimming cercariae were observed. Under favorable conditions of light and temperature fully developed cercariae may be seen emerging from the snails between the hours of 9 and 11 A.M. The body is usually ovoid but

almost finger-like when fully extended. The tail when extended is almost twice as long as the body and capable of considerable extension and contraction. Ten fixed specimens measured 0.23 to 0.28 mm in length by 0.13 to 0.17 mm in width. The tail varied in length between 0.39 and 0.52 mm. The whole body is opaque and packed with cystogenous cells. The excretory system consists of two trunks joined anteriorly and discharging posteriorly into the sides of the excretory bladder. The excretory tubes join to form a finger-like diverticulum below or to the side of the median eye-spot. A large number of fine granular excretory concretions, unequally distributed, are found in these tubes.

The Metacercaria (Fig. 9).—After the cercariae emerge from the snail they readily encyst on vegetation or on the surfaces of the containing vessel. The process of encystment is completed in 3 to 5 minutes. Ten cysts scraped from the surface of a stender dish on which they had formed 20 minutes previously varied in diameter from 0.16 to 0.18 mm. In side view they are hemispherical, the attached surface being flat. The metacercaria is infective soon after encystment and may remain so for at least 3 weeks if kept refrigerated.

Infection of Definitive Hosts.—Cysts, from naturally infected snails, formed on lettuce were fed to 4 ducks, 1 pigeon, 1 chicken, 1 rat, and 9 meadow mice but only the latter were successfully infected. The mice used in these experiments were kept in the laboratory for some time before they were fed cysts.

The first 2 mice that were fed cysts died three and eight days after infection. Both harbored large numbers of immature worms. Later 2 more mice were fed about 40 cysts apiece and after 10 days their fecal pellets were examined daily. In one filamentous eggs were detected 16 days after feeding the cysts. This mouse was sacrificed 23 days after feeding and found to have 10 adults in the cecum and 4 in the ileum. The other mouse was found to harbor 39 adult worms when it was autopsied 29 days after infection. These specimens appeared to be fully grown as eggs were found in the feces for 12 days prior to sacrificing the host. Five other mice were successfully infected and worms of different ages recovered. Specimens of the same species were also obtained from naturally infected muskrats at Douglas Lake, Michigan and Carlisle, Pennsylvania. Three specimens from the U. S. Nat. Mus. Helm. Coll., Nos. 28185, 40961, and 40981 taken from the cecum of muskrats and used by Harwood (1939) in his description of *Q. quinqueserialis*, were also available for comparison. No opportunity was presented to study the original material described by Barker and Laughlin as *Notocotylus quinqueserialis*.

Adult (Figs. 10, 12).—Because the earlier descriptions were neither made from experimental material nor from worms of known age additional data on this species is given with measurements of 20 whole mounts from the 29-day-infection in the meadow mouse.

Description: Medium sized, oval, muscular flukes, $2.91 \pm .29$ (2.53–3.51) mm long by $0.93 \pm .11$ (0.86–1.09) mm wide; anterior end more or less pointed, posterior end rounded; cuticle beset with minute spines on anterior half of ventral surface; oral sucker large, ventral, $0.34 \pm .04$ (0.21–0.43) mm in diameter; esophagus very short, bifurcating almost immediately; ceca irregular tubes descending to some distance below posterior median margin of testes with many dilations and indentations; genital pore ventral, posterior to intestinal bifurcation; five rows of ventral glands, number in lateral rows 16 to 19 with a mean of 17, in middle row 14 to 16 with a mean of 15; testes lobed, $0.48 \pm .05$ (0.41–0.60) mm long by $0.27 \pm .03$ (0.21–0.35) mm wide; cirrus eversible, echinate; ovary lobed, $0.28 \pm .03$ (0.22–0.34) mm long by $0.22 \pm .02$ (0.17–0.25) mm wide; vitellaria lateral, with about 12 to 17 groups of follicles on each side; position of vitellaria (length of body divided by forward extent of follicles) 1.9 to 2.2 with a mean of 2.1; uterus continues forward from the oötype in numerous transverse coils some of which extend beyond the ceca; uterine loops between vitellaria and cirrus sac on side of metraterm 4 to 7 with a mean of 6; seminal receptacle absent but replaced by a seminis receptaculum uterinum; excretory bladder receives 2 branched excretory tubules joined anteriorly below the oral sucker.

Only three described species resemble my material which has a large oral sucker and 5 rows of ventral glands. They are *Q. quinqueserialis* (= *Notocotylus quinqueserialis* Barker and Laughlin, 1911) from the muskrat and also from the meadow mouse (Harrah, 1922), *Notocotylus hassalli* McIntosh and McIntosh, 1934, from *Microtus pennsylvanicus* and from *Zapus hudsonius* (Erickson, 1938), and *N. wolgaensis* Skvortsov, 1935, from *Arvicola terrestris*. Comparisons indicate that my specimens were not *N. hassalli* because they have a smaller number and smaller clusters of vitelline follicles. Also they are not *N. wolgaensis* because it has the same number of ventral glands in each row. They were found to belong to *Q. quinqueserialis*.

The above description of *Q. quinqueserialis* agrees with the original except in a few points. The original stated that there are from 16 to 18 ventral glands in each row but in more than 80 specimens examined I have found the most common number of glands in the five rows to be 15 for the median and 17 for all the other rows. The next most common combination was 15 for the middle row and 18 for all the other rows. The highest total number counted in one specimen was 92 in which the middle row had 16 and all the other rows had 19. Large worms had about the same number of glands as smaller specimens. The original description also stated that the excretory tubules end blindly whereas they are found to join anteriorly below the oral sucker. The variations in body measurements are also greater in the original than in my material which was all of the same age. These differences are minor and can be explained as due to the small number and differently fixed specimens used for description. My material agrees with the redescription of *Q. quinqueserialis* given by Harwood (1939).

THE LIFE CYCLE OF *Notocotylus stagnicolae* n. sp.

The second species, *N. stagnicolae* n. sp., has a life history similar to

that of *Q. quinqueserialis*. In this case, however, the intermediate hosts are varieties of *Stagnicola emarginata* and the adults develop more readily in birds than in mammals.

The Eggs and Infection of Intermediate Hosts.—The eggs of *N. stagnicola* obtained from an experimental chicken are slightly larger than those of *Q. quinqueserialis*. The polar filaments are also longer (Fig. 23). Twenty fixed specimens measured 0.021 to 0.025 mm in length by 0.014 to 0.017 mm in width. The filaments varied in length from 0.50 to 0.62 mm.

On August 5, 1940, eggs from 84 worms, taken from an experimental chicken, were placed with about 200 laboratory-raised juveniles of *S. e. angulata*. A week after exposure some of these snails were examined. After this, examinations were made daily with few exceptions for a month, and then at longer intervals until the termination of the experiment 8 months after infection. Every one of 97 snails examined was infected with some larval stage of this species. *G. parvus* and *P. gyrina*, exposed to eggs of *N. stagnicola*, were found uninfected 23 days later.

No eggs were seen to hatch but infection probably occurred by penetration of the miracidia. The descriptions of all the larval stages except the daughter rediae and cercariae, which were studied from natural infections in *S. e. canadensis* and *S. e. angulata*, were made from material found in the examinations of these experimental snails. After 18 days the weather became much cooler and the lower temperatures in the laboratory probably retarded development considerably. Therefore, the time of appearance of the various stages and the sequence of development cannot be considered as normal in this experiment.

The Mother Sporocyst (Figs. 13–16).—The first mother sporocysts were found 7 days after infection. They were small spherical sacs about 0.035 mm in diameter, attached along the intestine by very thin membranous tissue. At this stage I could not distinguish any germ cells or developing embryos inside the sporocysts. The earliest embryo of a mother redia which consisted of about 8 cells was found 9 days after infection suspended in the cavity of a sporocyst 0.053 mm in diameter. In another sporocyst in the same snail a single developing redia was observed in which a pharynx could be made out. Many sporocysts each containing a single developing mother redia were found about 2 weeks after infection (Figs. 13–15). Eighteen days after exposure a well developed sporocyst (0.239 mm in diameter) was found containing a mother redia (Fig. 16) similar to those found free in the tissues along the intestine or in the digestive gland of the same snail. Sporocysts always contained only one redia and were found in the snails up to 33 days after infection. The heaviest reported sporocyst infection was 23, distributed throughout the mantle and along the intestine. Several sporocysts in other snails were found in the digestive gland.

The Mother Redia (Fig. 17).—As early as 14 days after infection and in most examinations for 8 months afterwards, mother rediae were found in the tissues of the experimental snails. At first they were located along the intestine but later they were all found in the digestive gland. The smallest free mother redia measured was 0.17 mm long by 0.08 mm wide; the largest 1.33 mm long by 0.19 mm wide. The number of free mother rediae in any one snail varied between one and 22 although one snail had 11 mother rediae and 15 mother sporocysts each containing one redia making a possible total of 27.

Mother rediae first showed food particles in the intestines 22 days after infection of the snails. The number and size of the food particles and the amount of pigmentation in the body wall increased as development progressed. In infections of 3 months or longer the mother rediae could be distinguished rather easily from the large number of daughter rediae by their larger size and deeper pigmentation. One large mother redia contained about 27 germ balls and another 15 daughter rediae and about 12 germ balls. Keeping the snails in the laboratory during the winter and feeding them bits of lettuce apparently produced conditions which brought about degeneration of daughter rediae. Besides an actively moving daughter redia one old mother redia contained the anterior ends of 2 daughters each consisting of a pharynx and a small portion of the body posterior to it. Why one of the rediae was apparently normal while 2 others in the same mother were degenerating is difficult to explain unless we suppose that the active redia had just matured and would degenerate later.

The Daughter Redia (Fig. 18).—Daughter rediae with pharynx and narrow intestinal lumen were first seen in the mother rediae of experimental snails 24 days after exposure. One of the larger developing daughter redia within the mother measured 0.37 mm in length by 0.17 mm in width. One of the first daughter rediae found free in the digestive gland of a snail 27 days after infection measured 0.389 by 0.194 mm. The number of daughter rediae in the experimental snails gradually increased. In one 45-day-infection there were 38; in 51 days about 50; in 75 days about 90; in 100 days about 100; in 195 days about 500. My estimate is that each mother redia produces at least 25 or 30 daughters. The large numbers of rediae found in the experimental snails was due to multiple infection.

Twenty-five mature fixed specimens of daughter rediae obtained from a natural infection in *S. e. angulata* varied in length from 0.70 to 1.15 mm and in width from 0.17 to 0.27 mm. The pharynx varied from 0.059 to 0.075 mm in diameter. The esophagus was short and measured between 0.011 and 0.041 mm in length. The intestine of mature specimens is filled with particles of various sizes and extends into the posterior third of the

body. The number of developing cercariae far enough advanced to show eye-spots is usually 3. The number of germ balls varies from 8 to 16. In several rediae the germ balls appeared to be concentrated in a funnel-shaped structure near the posterior end (Fig. 18). The most fully developed cercariae inside the rediae contained pigment material in the anterior end and an outline of the excretory trunks devoid of granules. The color of mature rediae is usually yellowish or brown.

The Cercaria (Fig. 19).—Notocotylid cercariae emerging from one specimen of *S. e. canadensis* collected from Walloon Lake, Michigan, were first suspected of being new for the region because they escaped late in the morning or shortly after noon. Their encystment was also delayed; in some cercariae the interval between emergence and cyst formation was as long as 20 minutes. Other observations on the course of the excretory tubules and measurements of body, tail, and cyst made it certain that this was a new species. Eleven additional infections of this type of cercaria were found in specimens of *S. e. angulata* collected in Burt Lake. Metacercariae from both collections were fed to experimental animals and identical adults recovered.

Eggs from some of these adults served as a source of infection for the experimental snails. Generally the digestive glands of these snails contained very few cercariae in spite of the length of the infection and the large numbers of rediae present. Five free swimming cercariae were recovered from one snail 75 days after exposure to infection. A normal daily cercarial output from a naturally infected snail is usually over 50.

The cercariae of *N. stagnicola* have 3 eye-spots when mature but in undeveloped cercariae the pigment is diffuse around the anterior end, with clumps in the area of the 2 lateral eye-spots. The bodies of 25 free swimming specimens fixed in Gilson's measured 0.32 to 0.57 mm in length by 0.08 to 0.21 mm in width. The tails varied in length between 0.62 and 0.94 mm or about twice as long as the body. The body is not as opaque as that of the cercaria of *Q. quinqueserialis*. The main parts of the excretory system consists of 2 trunks joined anteriorly below the median eye-spot and discharging posteriorly into the anterior sides of the excretory bladder.

The Metacercaria (Fig. 20).—Encystment sometimes occurred on the shell of the snail from which the cercariae were emerging although other surfaces are the usual sites for cyst formation. The time between emergence and cyst formation was from 5 to 20 minutes. Twenty-five cysts measured 0.20 to 0.25 mm in diameter with a mean of 0.23 mm. The encysted stage is infective as soon as fed to suitable definitive hosts. Cysts were also infective if kept in the refrigerator for at least a week.

Infection of Definitive Hosts.—Cysts formed from cercariae emerging from naturally infected snails were fed to 7 ducks, 7 chickens, 2 blue-winged

teals, 5 laboratory rats, 2 meadow mice, and 1 white mouse. A total of 283 worms of various ages were collected from the teals, ducks, and chickens. One immature specimen was found in the laboratory mouse and 4 worms in two of the rats.

The first successful infection resulted from feeding a young blue-winged teal; the second from feeding a merganser duckling; the third from feeding a laboratory-raised duckling. The 3-day-old worms recovered from the cecum of the blue-winged teal showed evidences of 2 remaining eye-spots and a beginning of the branching of the excretory trunks (Fig. 21). The 8-day-old worms taken from the merganser duckling were nearly mature and showed 3 rows of ventral glands. The 19-day-old worms taken from a laboratory-raised duckling appeared fully grown. The heaviest infection was obtained in a young rooster fed about 150 cysts. Seventeen days later 120 mature worms were recovered, of which 84 were found in one cecum and 36 in the other. All ducks and chickens which were fed cysts of this species yielded positive infections in the cecum.

Many mice and rats were fed cysts but only one mouse and 2 rats yielded worms, many immature. The results of feeding experiments with these animals indicate that they are abnormal hosts for this species. Since birds are the most suitable hosts it would be expected that wild waterfowl inhabiting the Douglas Lake region would be expected to be the natural hosts for this species. Eleven specimens of this form were taken from a semi-palmated plover, *Aegialitis semipalmata*, by Dr. Sterling Brackett at Douglas Lake.

The Adult (Figs. 22, 24, 25).—Twenty specimens of whole mounts taken from the cecum of an experimental chicken 13 days after infection were used in making the measurements for the following descriptions:

Notocotylus stagnicolae n. sp.

(Figs. 21–25)

Specific Diagnosis: Adult; characteristics of the genus; body elongate, concave ventrally, muscular, $2.99 \pm .18$ (2.67–3.40) mm long by $0.83 \pm .06$ (0.72–0.94) mm wide; anterior half of ventral surface covered with many spines arranged in oblique rows; oral sucker ventral, muscular, $0.16 \pm .008$ (0.13–0.18) mm; esophagus 0.09 to 0.18 mm in length, narrow, bifurcating anterior to opening of genital pore; intestinal ceca, irregular tubes descending to posterior median border of testes, with dilations and indentations; genital pore ventral, somewhat posterior to intestinal bifurcation; three rows of ventral glands which may be everted and in cleared condition usually show a slit in the surface; numbers in lateral rows from 14 to 17 with a mean of 15, in middle row 13 to 15 with a mean of 14; testes with 8 to 12 lobes along lateral margin, $0.44 \pm .04$ (0.34–0.56) mm long by $0.24 \pm .02$ (0.17–0.30) mm wide; distance of testes from posterior border $0.10 \pm .06$ (0.06–0.14) mm; cirrus echinate, eversible; both internal and external seminal vesicle present; ovary smaller than testes, $0.23 \pm .02$ (0.20–0.30) mm long by $0.26 \pm .02$ (0.21–0.30) mm wide, often lobed in outline; vitellaria lateral with about 24 to 34 groups of follicles on each side; position of vitellaria (length of body divided by forward extent of follicles) 1.8 to 2.1 with a mean of 1.9; from the oötype the uterus continues forward in numerous coils most

of which lie intracecal; uterine loops between vitellaria and cirrus sac on side of metraterm 3 to 5 with a mean of 4; seminal receptacle absent but lower portions of uterus containing numerous spermatozoa; excretory vesicle receiving 2 branched excretory tubules joined anteriorly below the oral sucker.

Hosts: *Gallus domestica*, *Anas domestica*, *Querquedula discors*, *Mergus merganser* (experimental), *Aegialitis semipalmata*.

Habitat: Cecum.

Locality: Douglas Lake, Michigan.

Type specimens: Co-types deposited in the U.S.N.M. Helm. Coll. No. 36839.

Studies in ventral gland relationships were made on 31 adult specimens of *N. stagnicolae* taken from the cecum of a chicken. It was noted that the glands usually showed up as indented oval crevices (Fig. 24). The most common alignment was 14 glands for the median row and 15 for each of the lateral rows. The next most common arrangement was 15 glands for the median row and 16 for each of the lateral rows. The highest total for the 3 rows was 48 of which 14 were found in the median row and 17 in each of the lateral rows. The lowest number observed for the median row was 13 and for the lateral rows 14. The number of glands in the middle rows anterior to the most anterior glands in the lateral rows also varied. Usually one but in many cases 2 glands in the median row were anterior to the most anterior glands in the other two rows.

The adults of *N. stagnicolae* can be differentiated from most other species of the genus *Notocotylus* by the following combination of characters: (1) only 3 to 5 uterine loops extending above the vitellaria; (2) the number of ventral glands in the lateral rows from 14 to 17, in the middle row from 14 to 15; (3) extent of vitellaria slightly anterior to midbody; (4) the number of lobes of testes along lateral border from 8 to 12; (5) measurements of reproductive organs. Because of insufficient descriptions of the adults of some species it is also necessary to use certain larval characters for differentiation.

In reviewing the literature I find 4 species which are so similar to *N. stagnicolae* that differentiation by the use of the adult characters listed above is very difficult. Of these *N. intestinalis* Tubangui, 1932, may be separated from *N. stagnicolae* by its very narrow ovaries and testes; *N. attenuatus* Rud. because the cercariae have a longer body and a shorter tail; *N. magnioratus* Yamaguti, 1934, because the rediae have posterior locomotor appendages; and *N. imbricatus* (Looss, 1896) by having cercariae which are smaller in size measurements.

These comparisons make it evident that no species has been described with characters identical in both larval and adult stages with *N. stagnicolae*. However, because of inconsistent bases of differentiation and incomplete descriptions it is hard to tell whether all the species described in the genus *Notocotylus* are valid and whether *N. stagnicolae* is really different from all of them. Subsequent life history studies may show that several of them are conspecific. Meanwhile, in the absence of better evi-

dence, it is considered preferable to keep the present species names in this genus and add *N. stagnicolae* as a new species.

DISCUSSION

It is interesting to note that the mother sporocyst of one species reported here, *Q. quinqueserialis*, contains four mother rediae and the sporocyst of the other, *N. stagnicolae*, contains only one. In the life cycle of *N. urbanensis*, described by the author in a preliminary note (Herber, 1940), mother sporocysts were found that contained both 6 and 8 mother rediae. Joyeux (1922) found 2 rediae inside the mother sporocyst in the species for which he worked out the life cycle. The rediae seen by Joyeux inside the mother sporocysts were probably mother rediae although he thought that there was only one generation of rediae in this life cycle. Several other workers, Looss (1896), Wesenburg-Lund (1934), and Yamaguti (1938), reported that they found 2 generations of rediae. The life cycle which Joyeux described is strikingly different from the others in this group since the cercariae have rudimentary tails and encyst without escaping from the snail intermediate host. All other cercariae described from this group have long tails, are active swimmers, and encyst on vegetation or on the snail shell after emerging from the intermediate host.

The theory that cysts of notocotylids need a certain time for maturing before they become infective was brought out by Harper (1929). The experiments reported here seem to indicate that metacercariae are infective immediately after encystment. Whether Harper's species is unusual in requiring such an interval cannot be determined without more experimental work.

The identification of adults of the subfamily NOTOCOTYLINAE and especially in the genus *Notocotylus* presents a great many difficulties because there is considerable similarity of structure and few good characters for differentiation. The following characters have been emphasized by various workers in separating them: size and shape of body, number and arrangement of ventral glands; size of organs, position and detailed structure of organs. Some of these characters are evaluated here in their relation to species differentiation.

Body size has very limited use as it is dependent upon several factors such as maturity, host relationship, state of contraction at the time of examination, and method of fixation. This character can only be used in separating species where variations are quite pronounced.

Ventral gland relationships are of some value as diagnostic characters. Since methods of fixing and mounting specimens may completely obscure them, the statement that they are absent in the description of a species should be viewed critically unless it is certain that proper methods of

study were used. Also, for the same reason the number and arrangement of these glands are often given incorrectly in species descriptions. The presence of 5 rows of these glands instead of 3 is of course a good character which gave the basis for the separation of the genus *Quinqueserialis* from *Notocotylus*. In my studies a considerable variation in number and arrangement was found which would have to be taken into consideration in comparing closely related species. It would seem, therefore, that the ventral gland relationship has a somewhat limited value in distinguishing closely related species of the genus *Notocotylus*.

The size of eggs has been used as a means of distinguishing species regardless of age or method of fixation. In my opinion egg-size is of little value as an aid in differentiating species but differences in length of egg filaments fixed soon after ovipositing are probably significant.

The outline of the testes of most species of *Notocotylus* is smooth along the median border but the lobing along the lateral borders can be used as a diagnostic character. Testes and ovary measurements can be used as aids in diagnosis only in a limited number of species and in series of worms of approximately the same age.

The number of uterine loops anterior to the vitellaria is dependent upon the range of the vitelline follicles. In most species the posterior limits of the vitellaria are immediately in front of the anterior limits of the testes but the forward extent varies because of the difference in the number of follicles. Therefore, the number of uterine loops anterior to the vitellaria and the position of the vitellaria are good diagnostic characters.

Larval characters have not been used extensively and have a limited value in distinguishing species of NOTOCOTYLINAE because of the small amount of work on life cycles. Body and tail size differences are often useful in distinguishing different species of cercariae. It has also been found that the relation of the anterior transverse portion of the excretory tubules to the median eye-spot is a very good diagnostic character. If the cercariae are allowed to encyst normally the diameter of such cysts is constant enough to use it as a means of distinguishing species. Size differences in body, tail, and cyst were utilized by L. and U. Szidat (1933) in distinguishing cercariae which when fed to suitable hosts produced different species of adults. Rothschild (1938) confirmed the value of such size differences in cercariae and also noted differences in the structure of the anterior transverse portion of the excretory tubules. The amount of pigmentation and time of encystment aids only as a sort of preliminary identification of cercariae. Rediae in this group usually have no posterior locomotor appendages and are much alike in the different species. In four species for which mother sporocysts are known they contain different numbers of rediae.

Certain of the earlier authors considered as separate species adults

found in different hosts. This is obviously unsound as one species may develop in a wide variety of animals. For example, *N. stagnicola*, a bird form, has developed to maturity in mammals. It was suggested by L. and U. Szidat (1933) that specificity is very marked in the case of the snail host. This view is confirmed in my life history studies. Although the degree of this specificity is not known at present, it is possible that it may have real significance.

SUMMARY

The life histories of *Quinqueserialis quinqueserialis* (Barker and Laughlin, 1911) and *Notocotylus stagnicola* n. sp. have been completed experimentally.

The definitive hosts of *Q. quinqueserialis* are *Ondatra zibethicus*, *Microtus pennsylvanicus*, and *Zapus hudsonius*. When eggs of this parasite were placed with the intermediate host, *G. parvus*, infection occurred and mother sporocysts could be recognized 9 days afterwards. Mother sporocysts of this species contain 4 mother rediae. About 20 days after exposure to infection daughter rediae were found in the digestive gland of the snails. About 23 days after infection immature cercariae emerged from the daughter rediae and migrated into the digestive gland tissues where a short period of maturation occurs. Mature cercariae were found 26 days after infection. They emerge from the snail and usually encyst on vegetation. Meadow mice become infected when fed metacercariae of this form. These flukes develop to maturity in about 2 weeks. Descriptions are given of all stages in the life cycle.

The definitive hosts of *N. stagnicola* n. sp. are *Gallus domestica*, *Anas domestica*, *Querquedula discors*, *Mergus merganser*, and *Aegialitis semipalmata*. Eggs of this parasite are infective when placed with the snail host, *S. e. angulata*. The course of the development in the intermediate host is very similar to that of *Q. quinqueserialis* except that the mother sporocyst of this form contains only one mother redia. In the definitive host these flukes mature in about 10 days. All stages of the life cycle of this trematode are also described.

The following characters were found to be most helpful in distinguishing different species of the NOTOCOTYLINAE: (1) the number of ventral glands in each row, (2) the anterior extent of the vitellaria, (3) the lateral lobation of the testes, (4) the body and tail size of cercariae, and (5) the course of the excretory tubules of cercariae. A discussion is given of the value of the different characters which have been used to differentiate the species of NOTOCOTYLINAE.

BIBLIOGRAPHY

- BARKER, F. D. AND LAUGHLIN, J. W. 1911 A new species of trematode from the muskrat, *Fiber zibethicus*. Tr. Am. Micr. Soc. 30: 261-274.
ERICKSON, A. B. 1938 Parasites of some Minnesota Cricetidae and Zapusidae,

- and a host catalogue of helminth parasites of native American mice. *Am. Midland Naturalist* 20: 575-589.
- HARPER, W. F. 1929 On the structure and life history of British freshwater larval trematodes. *Parasitology* 21: 189.
- HARRAH, E. C. 1922 North American monostomes primarily from freshwater hosts. *Illinois Biol. Monogr.* 7: 225-324.
- HARWOOD, P. D. 1939 Notes on Tennessee helminths. IV. North American trematodes of the subfamily Notocotylinæ. *J. Tennessee Acad. Sc.* 14: 421-437.
- HERBER, E. C. 1940 The mother sporocysts of three species of monostomes of the genus *Notocotylus* (Trematoda). *J. Parasitol.* 26 Suppl: 35.
- JOYEUX, CH. 1922 Recherches sur les notocotyles. *Bull. Soc. Path. Exot.* 15: 331-343.
- LOOSS, A. 1896 Recherches sur la faune parasitaire de l'Égypte. Première partie. *Mem. Inst. Egypt, Caire* 3: 1-252.
- LUTTERMOSER, G. W. 1935 A note on the life history of the monostome, *Notocotylus urbanensis*. *J. Parasitol.* 21: 456.
- MATHIAS, P. 1930 Sur le cycle évolutif d'un Trématode de la famille des Notocotylidae Lühe (*Notocotylus attenuatus* Rud.). *Compt. Rend. Acad. Sc.* 7 Jul.: 75-78.
- McINTOSH, A. AND McINTOSH, G. E. 1934 A new trematode, *Notocotylus hassalli* sp. nov. (Notocotylidae), from a meadow mouse. *Proc. Helm. Soc. Wash.* 1: 36-37.
- ROTHSCHILD, M. 1938 Notes on the classification of cercariae of the superfamily Notocotyloidea (Trematoda), with special reference to the excretory system. *Novitat. Zool.* 41: 75-83.
- SKVORTSOV, A. A. 1935 [Zur Kenntnis der Helminthfauna der Wasserratten (*Arvicola terrestris* L.).] *Vestnik Mikrobiol., Epidemiol. i. Parasitol.* 13: 317-326.
- SZIDAT, L. AND SZIDAT, U. 1933 Beiträge zur Kenntnis der Trematoden der Monostomidengattung *Notocotylus* Diesing. *Centr. Bakt.* 129: 411-422.
- SZIDAT, U. 1935 Weitere Beiträge zur Kenntnis der Trematoden der Monostomidengattung *Notocotylus* Diesing. *Centr. Bakt.* 133: 265-270.
- TUBANGUI, M. A. 1932 Trematode parasites of Philippine vertebrates. V. Flukes from birds. *Philippine J. Sc.* 47: 369-404.
- WESENBURG-LUND, C. 1934 Contributions to the development of the trematoda Digenea. Part II. The biology of the freshwater cercariae in Danish fresh waters. *Danske Vidensk. Selsk. Skr. Naturw. og Math. Afd.* 5: 1-223.
- YAMAGUTI, S. 1934 Studies on the helminth fauna of Japan. Part 3. Avian trematodes. II. *Japan. J. Zool.* 5: 557-560.
- 1938 Zur Entwicklungsgeschichte von *Notocotylus attenuatus* (Rud. 1809) und *N. magniozeatus* Yamaguti, 1934. *Z. Parasitenk.* 10: 288-292.

PLATE I

Stages in the life cycle of *Quinqueserialis quinqueserialis*
(All drawings made by use of camera lucida)

- FIG. 1. Mother sporocyst in mantle of snail, 16 days after exposure to infection.
FIG. 2. Mother sporocyst found free after crushing snail, 18 days after exposure to infection.
FIG. 3. Mother redia.
FIG. 4. Daughter redia.
FIG. 5. Cercarial embryo.
FIG. 6. Elongated cercarial embryo.
FIG. 7. Developing cercaria.
FIG. 8. Body and part of tail of mature cercaria.
FIG. 9. Metacercaria, encysted stage.
FIG. 10. Adult, ventral view.
FIG. 11. Egg.
FIG. 12. Outline of adult showing arrangement of ventral glands.

PLATE II

Stages in the life cycle of *Notocotylus stagnicolae* n. sp.
(All drawings made by use of camera lucida)

- FIG. 13. Mother sporocysts showing attachment along edge of snail intestine, 14 days after exposure.
FIG. 14. Mother sporocyst showing young mother redia, 16 days after exposure.
FIG. 15. Mother sporocyst showing almost mature mother redia, 16 days after exposure to infection.
FIG. 16. Mother sporocyst showing mature mother redia ready to break through the wall of mother sporocyst, 18 days after exposure to infection.
FIG. 17. Mother redia, 57 days after exposure.
FIG. 18. Redia containing cercariae, natural infection.
FIG. 19. Mature cercaria.
FIG. 20. Encysted metacercaria.
FIG. 21. Immature adult, 3 days after feeding.
FIG. 22. Adult, ventral view.
FIG. 23. Egg.
FIG. 24. Outline of adult showing arrangement of ventral glands.
FIG. 25. Outline of posterior ends of three adults, showing lobation of testes.

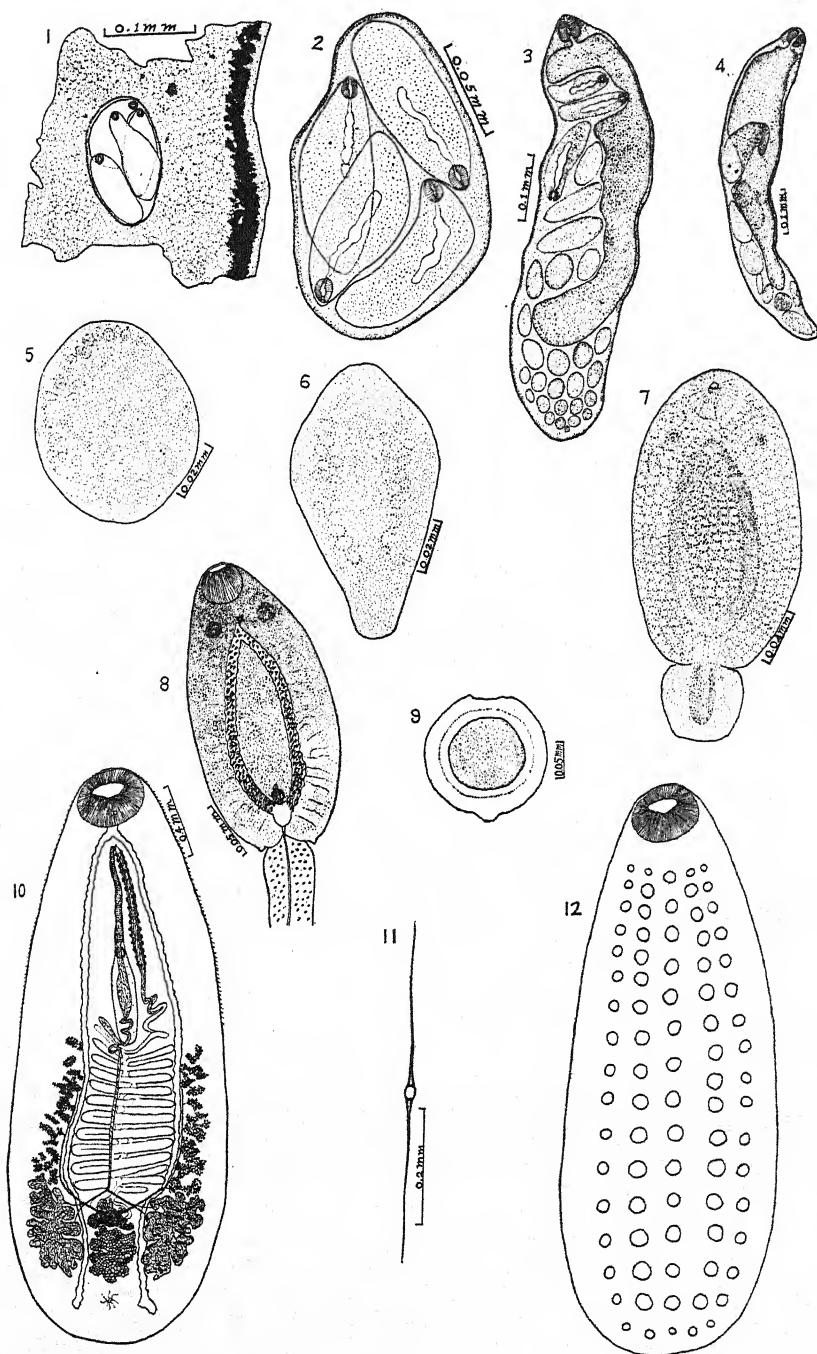


PLATE I

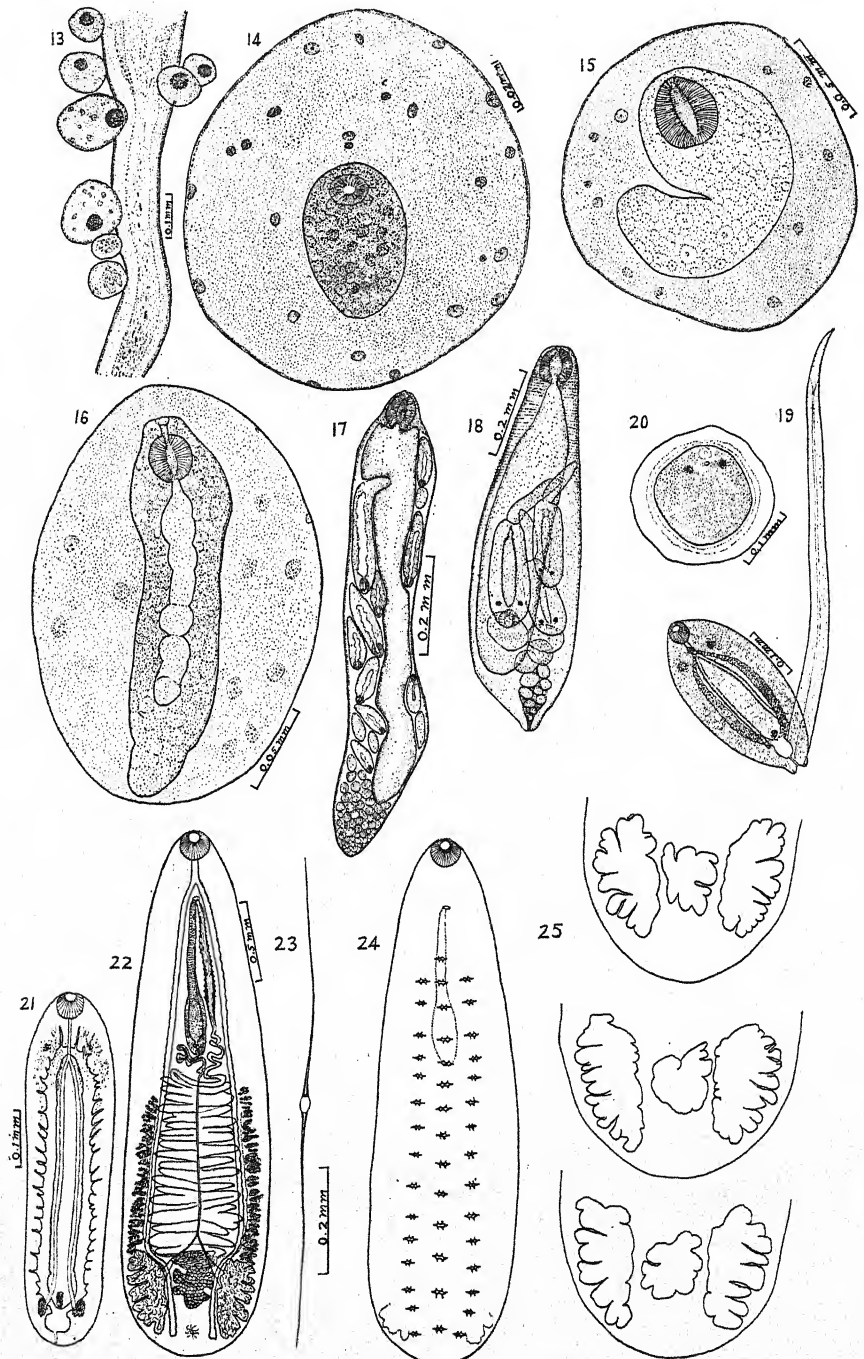


PLATE II

ACTIVE IMMUNITY IN MICE AGAINST *TRICHINELLA SPIRALIS*

JAMES T. CULBERTSON

Department of Bacteriology, College of Physicians and Surgeons,
Columbia University, New York

The acquisition of immunity against *Trichinella spiralis* through recovery from a prior infection with the homologous parasite has been demonstrated in the rat (1), the guinea pig (2), the pig (3), and the monkey (4). Most investigators are agreed that the acquired immunity is directed largely against the intestinal phase of the parasite, although the precise mechanism of the immunity is still obscure. Some believe the immune response is made chiefly or entirely by the tissues of the intestinal wall itself, and that the tissues of the remainder of the body have comparatively little or nothing to do with the immune process. An increased secretion of mucus and increased intestinal peristalsis are presented by those supporting this view as the chief factors operative in the immunity. These workers generally have failed to demonstrate either the passive transfer of immunity with the serum or the development of an inflammatory reaction in the intestinal wall of the immune host, and much of their contention is based upon these negative observations (5). Other investigators, however, consider that a generalized immune response must be made to the parasite before an animal can acquire a substantial degree of immunity, although they feel that this response may, and probably does, act upon the parasite only in strategically disposed sites, of which the intestine is one.

In the present paper, the results are offered from a study of acquired immunity to trichiniasis in the albino mouse. Strangely, the mouse has been but little employed in experimental trichiniasis. One of the few reports in which this animal has been used is that by the author and Kaplan of some years ago in which it was shown that rabbit antiserum rich in the specific antibody conferred to mice a partial immunity against trichiniasis (6). It is now shown that mice become actively immune to *Trichinella spiralis* as a result either of prior infection with the parasite or of vaccination with its antigens. The results are offered with a view to throwing some further light upon the mechanism of immunity in this disease.

GENERAL METHODS

The Method of Infecting Mice.—The larval worms were obtained from the muscles of infected rats, by the method to be described presently. After the larvae were freed of all rat tissue, they were suspended in bac-

Received for publication, July 4, 1941.

teriological nutrient broth to which 20 per cent of gelatin was added. In this medium, the worms not only survived for several days, but also remained in relatively stable suspension for some minutes, thus facilitating the administration of approximately identical doses of larvae to each mouse of a series being infected. Known numbers of worms, usually in a fluid volume of 0.1 cc, were delivered by syringe through a blunted 22-gauge hypodermic needle lowered carefully into the retropharynx of each mouse. Usually the mice swallowed the fluid, and with it the worms, with no trouble.

The Recovery of Adult Worms from the Intestine.—From four to seven days after larvae were fed to certain of the mice, the animals were killed with ether and autopsied. The small intestine was placed in physiological salt solution and slit lengthwise with small scissors. The intestine was then agitated so as to free the living, moving worms into the suspending fluid. The whole of this fluid was examined at once for the presence of adult worms, small portions being transferred to Syracuse watch glasses and searched with a binocular dissecting scope ($\times 7$, $\times 20$). The intestine was agitated in fresh amounts of salt solution as required, search being continued until no further worms were recovered.

The Recovery of Larval Worms from Muscle Tissue.—From 27 to 35 days after infection, the animals were killed with ether, skinned, and eviscerated. The remaining carcass was then ground in an ordinary meat grinder. The ground tissue was weighed and suspended in 0.5 per cent hydrochloric acid (at 37°C) to which 1.0 per cent powdered pepsin was added. Thirty cubic centimeters of this fluid were used per gram of mouse tissue. The mixture was placed in an incubator at 37°C and agitated constantly until all the meat was digested (usually within four hours). When digestion was complete, samples of the fluid were transferred to Syracuse watch glasses and the number of larval worms determined by direct count with the aid of a low-power binocular microscope ($\times 7$, $\times 20$). Finally, the number of worms in the total fluid volume, and thus in the entire carcass, was established by calculation.

THE ACQUISITION OF IMMUNITY THROUGH PRIOR INFECTION

Mice were fed one or more preliminary doses of living *Trichinella* larvae, and then, after an interval, were fed, together with control mice, a test dose of larvae. Some of the animals were autopsied after from five to seven days, and the adult worms in the intestine were recovered. Other mice were let live for from 27 to 35 days. The number of larval worms recoverable from the muscle tissue of these animals was then determined.

The Development of Adult Worms in the Intestine.—Two groups of mice were used to determine whether or not prior infection with *Trichi-*

nella prevented larval worms fed subsequently from developing to adults. Group A (5 mice) had received 100 larvae 22 days before the test feeding of 175 larvae. Group B (6 mice) had received 100 larvae 60 days before and 500 larvae 30 days prior to the test feeding of 250 larvae. The animals of Group A, with 7 control mice, were autopsied 5 days, and those of Group B with 5 controls 7 days, after the test feeding.

In the case of none of the previously infected mice were so many adult worms recovered as were found in the control mice. From the 5 previously infected animals of Group A, 1, 3, 4, 23, and 45 worms were recovered, representing an average of 8.6 per cent of the larvae fed. From Group B, 0, 0, 1, 1, 2, and 17 adults were recovered, representing 1.4 per cent of the worms fed. From the control mice for Group A, in contrast, 65, 82, 83, 101, 102, 108, and 144 (averaging 55.8 per cent) and, from those of Group B, 61, 96, 137, 146, and 197 (averaging 50.8 per cent) of the larvae fed were recovered as adult worms.

The Presence of Larval Worms in the Muscles.—In order to test for the effect of previous infection upon the amount of muscle invasion by larval *Trichinella*, a group of 17 mice were fed four doses each of 250 larvae, given, respectively, 60, 36, 22, and 10 days before a test dose of 1000 larvae. Nine control mice were also fed the test dose. All the mice which survived the test dose, including controls, were killed from 27 to 35 days after infection, and the number of larval worms in the muscles determined.

The muscles of the control mice harbored individually 110,250, 117,605, 119,475, 129,724, 152,530, 163,213, 174,440, 180,964, and 243,436 larvae, or on the average, 154,626 larvae. The muscles of the previously infected group in contrast, yielded 12,768, 16,966, 25,979, 39,712, 62,216, 101,500, 161,468 larvae, or, on the average, 60,087 larvae. Thus, the controls suffered approximately two and one-half times as much muscle invasion as the previously infected animals, in spite of the fact that the total number of infective larvae fed the controls was but one-half that fed the "immunized" mice. Indeed, the number of larvae recovered from the immunized group is only little more than the number expected as a result of the initial immunizing dose of 250 larvae. (Compare average of 60,087 for these immune mice with 55,139 for control mice for the vaccinated animals, which were given 250 larvae.) Thus, following the initial dose, these mice must have been almost perfectly protected against muscle invasion.

By the third day subsequent to the test dose, many of the previously infected mice revealed an exudate coming from the anus. When this exudate persisted for several days, death occurred. Altogether, 10 of the 17 previously infected mice died, the deaths occurring from the 9th to 23rd day after the test infection. Not any of the control mice pre-

sented this symptom, and none died during this period. On the other hand, the surviving mice of the previously infected group, continued to add weight, following an initial decline immediately after the test feeding, and appeared to suffer not at all from the infection. The control mice, in contrast, lost little or no weight until during the fourth week after the test dose. When autopsied, these animals were rapidly losing weight and some were moribund. The ultimate survival of any of the control mice was unlikely.

THE ACQUISITION OF IMMUNITY THROUGH VACCINATION

Earlier workers have endeavored to protect animals against *Trichinella spiralis* by vaccination with the antigens of the homologous parasite. Rats can generally be protected by such vaccination (7), although efforts thus to protect hogs have failed (3). The effect of parenteral vaccination upon the resistance of mice was tested in an effort to determine whether immunity could be developed in this animal in the absence

TABLE 1.—Summary of data. Average number of adult or larval *Trichinella spiralis* recovered from immunized and from control mice

No. of mice used	Method of immunization	No. of larvae fed as test dose	Days till autopsy after test dose	Average No. of adults recovered from intestine	Average No. of larvae recovered from muscles
5	Prior infection (a)	175	5	15.2	—
6	" (b)	250	7	3.5	—
7	Control	175	5	95.0	—
5	"	250	7	127.4	—
7	Prior infection (c)	1000	27-35	0.0	60,087
9	Control	"	"	0.0	154,626
11	Vaccination (d)	250	4	71.5	—
10	Control	"	"	132.0	—
9	Vaccination (d)	250	29	0.0	20,248
7	Control	"	"	0.0	55,139

(a) 100 larvae fed 22 days before test infection.

(b) 100 larvae fed 60 days, and 500 larvae fed 30 days before test infection.

(c) 250 larvae fed 60, 36, 22, and 10 days before test infection.

(d) Eight injections, given on alternate days, of one-tenth cc of 1 per cent suspension of *Trichinella spiralis* powder in carbolyzed salt solution per 10 gm body weight.

of previous intestinal infection with this parasite. The effect both on the development of adult worms in the intestine of previously vaccinated mice and upon the larval invasion of their musculature was determined.

The mice were vaccinated according to the following procedure: one-tenth cc of 1 per cent suspension of *Trichinella spiralis* powder in carbolyzed salt solution was administered per 10 gm mouse body weight. Eight injections were given on alternate days.

The Development of Adult Worms in the Intestine.—Five days after the last injection of vaccine, eleven mice, along with ten control animals, were fed 250 larvae. Four days later, all the animals were autopsied, and their small intestines searched for adult *Trichinella*.

The vaccinated mice yielded only about half as many worms as did the normal mice. From the vaccinated mice, 29, 30, 34, 35, 43, 50, 85, 88, 90, 125, and 178 adults were recovered. From the controls, 55, 103, 105, 113, 116, 120, 123, 166, 188, and 231 adults were recovered. Expressed as percentages of the larvae fed, the average yields by the vaccinated and control groups were, respectively, 28.4 per cent and 52.8 per cent. Two of the eleven vaccinated animals had, evidently, failed to respond to vaccination, but all of the nine remaining mice acquired a significant degree of immunity.

The Presence of Larval Worms in the Muscles.—To determine the relative amount of muscle invasion with larval *Trichinella* in vaccinated and control mice, 9 vaccinated and 7 normal animals were fed 250 larvae on the fifth day after the last administration of vaccine. After from 28 to 29 days, all the mice were autopsied and their muscles digested, as described earlier.

The nine vaccinated mice yielded 4,290, 6,396, 11,040, 11,647, 14,840, 17,189, 26,880, 44,800, and 45,156 larvae—an average of 20,248 larval worms. The control animals, in contrast, yielded 23,545, 49,800, 56,832, 57,426, 57,456, 59,640, and 81,280 larvae—an average of 55,139 larvae. Most of the vaccinated mice were rather highly immunized, apparently, as a result of vaccination, although two animals yielded almost as many larval worms as the control mice.

DISCUSSION

From the data offered in this paper, it is clear that immunity is engendered by mice against *Trichinella spiralis* both after prior infection with this parasite and after vaccination with its antigens. It is particularly noteworthy that, after immunization by either method, the immunity is effective against the intestinal phase of the parasite. The same finding has been reported previously after passively immunizing mice against this parasite (6). As may be expected, however, a higher level of immunity is developed by infection with *Trichinella* than by vaccination or by passive immunization. However, this quantitative difference by no means implies that the immune response after infection is qualitatively distinct from that after the two less effective procedures.

One matter of considerable interest in the results offered is the death, a few days after the test dose, of some of the mice immunized by prior infection. These animals suffered acutely from the test dose beginning about three days after its administration. The control animals at this time were not noticeably affected. Evidently the immune animals manifested a hypersensitive response to the *Trichinella* substance. The presence of an exudate in the intestine of the immune mice during these days supports this view, suggesting that an inflammatory response had been

initiated. Unfortunately, however, the response to the infection became so violent that many of the mice succumbed. Similarly, acute early symptoms, suggestive of hypersensitivity to the *Trichinella* substance, have also been observed in cases of human trichiniasis which have suffered reinfection (8). The capacity of highly immune animals to make such dangerous or even fatal hypersensitive responses is, of course, well known in tuberculosis and many other infectious diseases.

SUMMARY

Mice become partially immune to *Trichinella spiralis* as a result of prior infections with the specific parasite. They likewise manifest an immune response to this form after repeated vaccination with the antigens of the homologous worm. The immunity against *Trichinella* is directed particularly against the intestinal phase of the parasite, no matter whether the immune response is engendered by prior infection or by vaccination.

BIBLIOGRAPHY

1. DUCAS, R. 1921. L'immunité dans la trichinose. These. Paris. Jouve et Cie.
- McCoy, O. R. 1931 Infection of rats with *Trichinella spiralis*. Am. J. Hyg. 14: 484-494.
- BACHMAN, G. W. AND OLIVER GONZALEZ, J. 1936 Immunization in rats against *Trichinella spiralis*. Proc. Soc. Exper. Biol. & Med. 35: 215-217.
2. ROTH, H. 1939 Experimental studies on the course of trichina infection in guinea pigs. Am. J. Hyg. 30: 35-64.
3. BACHMAN, G. W. AND RODRIGUEZ-MOLINA, R. 1933 Resistance to infestation with *Trichinella spiralis* in hogs. Am. J. Hyg. 18: 266.
4. MCCOY, O. R. 1932 Experimental trichiniasis infection in monkeys. Proc. Soc. Exper. Biol. & Med. 30: 85-86.
5. MCCOY, O. R. 1940 Rapid loss of *Trichinella* larvae fed to immune rats and its bearing on the mechanism of immunity. Am. J. Hyg. 32: 105-116.
6. CULBERTSON, J. T. AND KAPLAN, S. S. 1938 A study upon passive immunity in experimental trichiniasis. Parasitology 30: 156-166.
7. MCCOY, O. R. 1935 Artificial immunization of rats against *Trichinella spiralis*. Am. J. Hyg. 21: 200-213.
8. BERCOVITZ, Z. 1941 Personal communication.



PASSIVE TRANSFER OF IMMUNITY TO *TRICHINELLA SPIRALIS* IN THE RAT

JAMES T. CULBERTSON

Department of Bacteriology, College of Physicians and Surgeons,
Columbia University, New York

Rats have been shown to acquire immunity against *Trichinella spiralis* after a preliminary infection with this parasite (1) or after vaccination with its antigens (2). The attempts thus far to demonstrate passive immunity in the rat, however, have failed, although antibody has been detected in the serum of immune rats (3), and larval worms immersed in such serum generally lost their infectivity (4). It has also been shown that immune mother rats transfer immunity to their young (5), although the precise route of transfer and the significance of the serum antibody in such transfer is not as yet established.

The present paper offers results from a study designed to test for passive transfer of immunity to *Trichinella spiralis* in the rat, using the serum of immune rats. The tests have been performed in the 10-day-old nurslings of normal mother rats.

GENERAL METHODS

Methods of infection of animals, recovery of adult *Trichinella* from the intestine, and recovery of larvae from muscle were the same as used in previous work with mice (6). For the preparation of immune serum, four adult normal rats were fed 2000 isolated larvae of *Trichinella spiralis*. After one month, these animals were bled and their serums pooled for use in the present work. Several normal animals were bled to provide the normal serum with which some of the control rats were injected. Five-tenths cubic centimeter of these serums were administered intraperitoneally on the two days prior to and on the day following infection. The dose given represented 3 cc per 100 gm weight of the recipient.

RECOVERY OF ADULT *Trichinella* FROM THE INTESTINES OF PASSIVELY IMMUNIZED RATS AND FROM CONTROL RATS

The first experiment was designed to determine whether the administration of an immune serum to an animal affected the development of the larval worms fed to this animal. Twenty-one 10-day-old rats nursing normal mothers were used in this study. Eight were injected with immune serum, and 7 with normal serum according to the schedule previously described. These rats, together with the six remaining animals which were given no serum, were then each infected with 250 larvae.

Received for publication, July 4, 1941.

Four days after infection, all the animals were killed with ether and their small and large intestines searched for adult *Trichinella*.

The rats which were injected with immune serum yielded distinctly fewer adult *Trichinella* than did those given normal rat serum, or those kept as untreated controls. Those given immune serum yielded 3, 5, 7, 8, 9, 16, 16, and 26 adult worms. Those given normal serum yielded 17, 40, 65, 66, 71, 79, and 83 adults. Those given no serum yielded 38, 44, 52, 73, 78, and 86 adult worms. On the average, only 4.5 per cent of the larvae fed were recovered as adults from the passively immunized group, whereas 24.0 per cent and 24.7 per cent were recovered, respectively, from the group given normal serum and from the untreated group.

RECOVERY OF LARVAL *Trichinella* FROM THE MUSCLES OF PASSIVELY IMMUNIZED RATS AND FROM CONTROL RATS

The second experiment was designed to determine the number of larval *Trichinella spiralis* in the muscles of immunized rats compared with control animals. Fourteen 10-day-old rats nursing two normal mothers were used for this work. Seven of these were given immune rat serum, and four were given normal serum intraperitoneally according to the previously described schedule. The three remaining rats were given no serum. Each of the animals was fed 250 isolated larvae. Four weeks after infection the rats were killed with ether, skinned, and eviscerated. The remaining carcass was digested and the total larval yield determined.

Fewer larvae were obtained from the muscles of passively immunized rats than from the control animals. From those given immune serum, 3,125, 6,234, 6,318, 13,248, 14,458, 18,414, and 22,830 larvae were recovered. From those given normal rat serum 36,630, 46,189, 49,369, and 58,266 larvae were obtained. From those given no serum 26,964, 51,129, and 91,600 larvae were found. On the average, 12,089 larvae were recovered from the muscles of the animals given immune rat serum, whereas from those given normal serum and from the untreated controls the average yields were, respectively, 47,613, and 56,564.

TABLE 1.—Summary of data. Average recovery of adult *Trichinella* from intestine and larvae from muscles of passively immunized mice and from controls

No. of rats in group	Rat serum given	Days till autopsy after test dose	Average No. of adults recovered from intestine	Average No. of larvae recovered from muscle
8	Immune	4	11.2	—
7	Normal	4	60.0	—
6	None	4	61.7	—
7	Immune	28	0	12,089
4	Normal	28	0	47,613
3	None	28	0	56,564

Test dose of larvae: 250 isolated larvae.

Administration of serum: 0.5 cc intraperitoneally for two days before, and one day following infection.

All rats 10 days old at start of experiment.

DISCUSSION

The results presented indicate that the immunity actively acquired by rats against *Trichinella spiralis* can be passively transferred to normal rats by transfer of the serum. The passively immunized animal evidently checks the infection in the same manner as does the actively immunized rat—namely, by limiting the development of adult worms in the intestine. This developing stage of the parasite is evidently the one most vulnerable to the immune effect. The limitation upon development is less nearly perfect in passively immunized animals than in animals immunized by previous infection, as would be expected. The comparatively small amount of serum transferred (3 cc per 100 gm by body weight on three days)—especially following the dilution which this experiences in the body of the recipient—could hardly equal in its effect upon *Trichinella* that of the total blood of an animal actively immunized by prior infection. Nevertheless, there is no reason to suspect that this difference in effect has a qualitative basis.

In the present experiments, the demonstrated immunity is undoubtedly conferred by some substance in the transferred serum. This humoral substance—which is perhaps antibody—would certainly be as effective in the animal which developed it as it is after transfer to a normal animal. Therefore, the same humoral immune mechanism operative after passive transfer must act likewise in actively immunized animals and be at least in part responsible for their acquired immunity. In both actively and passively immunized animals, there is ample opportunity for the humoral substance to come in contact with the developing parasite, since the larval *Trichinella* characteristically bores between the villi and into the intestinal glands to feed on the intestinal mucosa (7).

It is felt by some that the immune response in trichiniasis is made so promptly that the usual (humoral antibody) defense mechanism could hardly be responsible. It is pointed out that actively immunized rats expel *Trichinella* larvae in from 3 to 18 hours after these are ingested (8). Yet, it should be remembered that an Arthus reaction becomes evident in the skin of a well sensitized rabbit within two or three hours after a specific antigen is injected, even if the animal be passively sensitized (9). Furthermore, fatal anaphylactic shock may occur within a few minutes after the homologous antigen is injected into a well-sensitized guinea pig, again, no matter whether the animal be actively or passively sensitized. Finally, allergic reactions in the skin as well as other tissues (e.g., intestine) of man are typically immediate responses, reaching their peak within a few minutes after the tissue has had contact with the specific antigen. Thus the rapidity of the immune response in trichiniasis is not without precedent in immunology, and the conventional mechanisms, cannot, therefore, be excluded as the basis of immunity in this infection.

CONCLUSIONS

Immunity to *Trichinella spiralis* can be passively conferred upon normal rats by transferring to them serum from a rat immunized by prior infection. As might be expected, the immune response of passively immunized rats is less powerful than that generally manifested by actively immunized animals.

BIBLIOGRAPHY

1. DUCAS, R. 1921 L'immunité dans la trichinose. These. Paris. Jouve et Cie.
McCoy, O. R. 1931 Immunity of rats to reinfection with *Trichinella spiralis*.
Am. J. Hyg. 14: 484-494.
- BACHMAN, G. W., AND OLIVER-GONZALEZ, J. 1936 Immunization in rats against *Trichinella spiralis*. Proc. Soc. Exper. Biol. and Med. 35: 215-217.
2. MCCOY, O. R. 1935 Artificial immunization of rats against *Trichinella spiralis*.
Am. J. Hyg. 21: 200-213.
3. OLIVER-GONZALES, J. 1940 The in vitro action in immune serum on the larvae and adults of *Trichinella spiralis*. J. Infect. Dis. 67: 292-300.
4. MAUSS, E. A. 1940 The in vitro effect of immune serum upon *Trichinella spiralis* larvae. Am. J. Hyg. 32D: 80.
5. MAUSS, E. A. 1940 Transmission of immunity to *Trichinella spiralis* from infected animals to their offspring. Am. J. Hyg. 32D: 75.
6. CULBERTSON, J. T. 1942 Active immunity in mice against *Trichinella spiralis*.
J. Parasitol. 28: 197-202.
7. HELLER, M. 1933 Entwickelt sich die *Trichinella spiralis* in der Darmlichtung ihres Wirtes? Z. Parasitenk. 5: 370-392.
8. MCCOY, O. R. 1940 Rapid loss of *Trichinella* larvae fed to immune rats and its bearing on the mechanism of immunity. Am. J. Hyg. 32D: 105-116.
9. CULBERTSON, J. T. 1935 The relationship of circulating antibody to the local inflammatory reaction to antigen (the Arthus phenomenon). J. Immunol. 29: 29-39.

AGE RESISTANCE OF THE ALBINO RAT TO *CYSTICERCUS FASCIOLARIS*

SYLVIA H. GREENFIELD*

Department of Bacteriology, College of Physicians and Surgeons,
Columbia University, New York

Although age resistance has been demonstrated in many protozoan infections as well as in infections with a number of nematodes, this phenomenon has been observed with only very few cestode parasites. It has been described for *Hymenolepis nana* in man (1), as well as for its rodent variant, *Hymenolepis fraterna*, in rats and mice (2). The chicken is known to manifest age resistance to *Railletina cesticillus* (3), as is also the herring gull to a species of *Diphyllbothrium* (4). The development of natural resistance with age has likewise been suggested for the rat against *Cysticercus fasciolaris* (5), the larval stage of the cat tapeworm *Taenia taeniaeformis*.

The possibility of the manifestation of age resistance by rats to *Cysticercus fasciolaris* has been reinvestigated by the author, and the results of this study are offered in the present paper. It has been found that older rats rarely develop significant infections with this parasite, whereas younger animals—if past the weaning age—suffer heavy infections. However, nursing young, even if born of normal mother rats, are like old rats comparatively or wholly refractory to this parasite.

GENERAL METHODS

The infection and examination of rats.—Rats (Sherman strain) of known age were infected by mouth with 500 onchospheres of *Taenia taeniaeformis* obtained by opening a gravid segment of the parasite collected freshly from a laboratory cat. The onchospheres, suspended in 0.1 cc of physiological salt solution, were delivered by syringe through a blunted 22-gauge hypodermic needle lowered carefully into the retropharynx of each animal. The rats were autopsied 28 days after infection, and the number of living and of dead cysticerci counted on the surface of their livers.

PROCEDURE AND RESULTS

Altogether 144 rats of age groups ranging from newly born to 11 months old animals were used in this experiment. Approximately equal numbers of animals of each sex were included in the different groups.

Received for publication, July 4, 1941.

* The author wishes to express her appreciation to Dr. James T. Culbertson, of the Department of Bacteriology, College of Physicians and Surgeons, under whose direction these experiments were performed, for his many helpful suggestions and kind cooperation.

The results of the cyst counts made at the autopsy of 54 representative animals, all of which were infected simultaneously from the same suspension of onchospheres, are presented in Table 1, and photographs of the

TABLE 1.—Number of cysticerci counted in rats when autopsied 4 weeks after infection per os with 500 onchospheres of *Taenia taeniaeformis*

Rat number	Sex	Age when infected	Number of cysts recovered		
			Living	Dead	Total
1-4	♂+♀	New-born	0	0	0
5-8			0	0	0
9-11	♂+♀		0	0	0
12	♂+♀		0	1	1
13	♂+♀	7 days	0	3	3
14-16	♂+♀		0	0	0
17	♂+♀		0	1	1
			0	0.6	0.6 Av.
18	♂+♀		3	64	67
19	♂+♀		0	41	41
20	♂+♀	15 days	0	73	73
21	♂+♀		1	52	53
22	♂+♀		38	70	108
23	♂+♀		26	117	143
			11.2	69.5	80.8 Av.
24	♂+♀		103	0	103
25	♂+♀		185	0	185
26	♂+♀	25 days	208	0	208
27	♂+♀		126	0	126
28	♂+♀		110	0	110
29	♂+♀		195	0	195
			154.5	0	154.5 Av.
30	♂+♀		13	130	143
31	♂+♀	45 days	64	36	100
32	♂+♀		77	5	82
33	♂+♀		73	0	73
			56.7	42.7	99.5 Av.
34	♂+♀		80	82	162
35	♂+♀	60 days	34	126	160
36	♂+♀		5	83	88
37	♂+♀		16	64	80
38	♂+♀		7	59	66
39	♂+♀		44	52	96
			31	77.6	108.6 Av.
40	♂+♀	4 mos.	0	141	141
41	♂+♀		13	77	90
42	♂+♀		27	123	150
43	♂+♀		0	11	11
			10	38	48 Av.
44	♂+♀		0	28	28
45	♂+♀	6 mos.	0	58	58
46	♂+♀		0	17	17
47	♂+♀		0	27	27
			0	32.5	32.5 Av.
48	♂+♀		0	50	50
49	♂+♀		0	59	59
50	♂+♀	11 mos.	0	151	151
51	♂+♀		0	29	29
52	♂+♀		0	39	39
53	♂+♀		0	17	17
54	♂+♀		0	28	28
			0	53.2	53.2 Av.

isolated liver lobes of a single representative of the different age groups are shown in Fig. 1.

The rats 25 days old when infected were most susceptible to this parasite. Many cysts developed in these animals and essentially all were

living when the host was autopsied. In rats of older groups, progressively fewer cysts developed, and, of these, only part were alive when the host was autopsied. No living cysts whatsoever were recovered from rats six months old or more when infected.

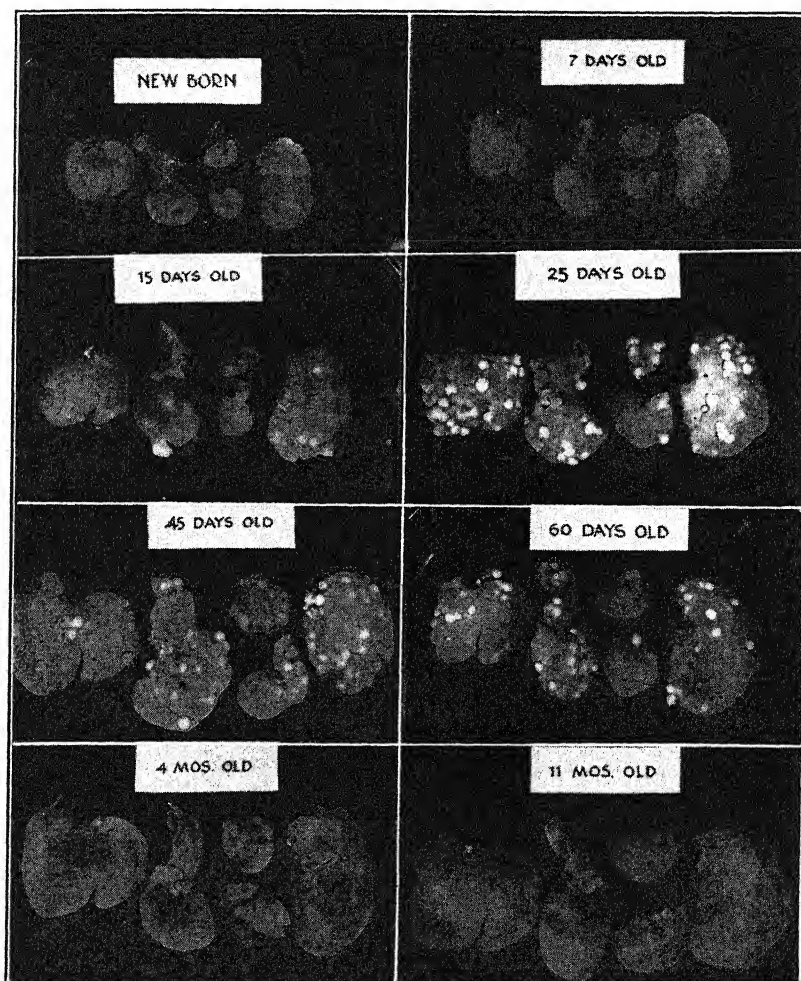


FIG. 1. Photographs of liver lobes of representative rats of the designated age groups autopsied four weeks after being fed 500 onchospheres of *Taenia taeniaeformis*, showing the number of *Cysticercus fasciolaris*.

Among the rats of groups less than 25 days old when infected, progressively fewer cysts were observed as the age group of the host decreased. Rats infected on the day of birth revealed at autopsy 4 weeks later no cysts whatsoever. A few dead cysts but no living ones were found when rats seven days old at infection were autopsied. A number

of living cysts and many dead ones were found in the rats infected at 15 days of age. The mothers of these animals were also autopsied and found to be uninfected.

DISCUSSION

The results given indicate that rats 25 days old are most susceptible to infection with *Cysticercus fasciolaris*. This high susceptibility is preserved largely until the animals reach two months of age. Thereafter, however, the natural resistance of rats increases markedly and those four months old or more usually prevent wholly the maturation of cysticerci, even though the parasite establishes itself initially in the livers of these animals. Curtis, Dunning, and Bullock (5) observed that the percentage of animals which was successfully infected varied significantly with the age at which the onchospheres were administered. From experiments carried out on large numbers of animals, using standard suspensions of *Taenia* eggs, they found that 76% of rats one month old were susceptible to infection, while only 19% of those four months of age showed cysts. However, under certain circumstances, mature cysts may develop in older animals. Miller (6) has reported, in a small group of albino rats 170-172 days of age when infected, an average of 22 living and 19 dead cysts. A heavy suspension of 900 onchospheres was fed in this case because a large number of the eggs were immature. Although mature cysts were present in these animals over five months of age, there is no means of evaluating the significance of such results in view of the fact that data upon animals of other age groups infected with a similar suspension is not available.

The remarkable resistance of the nursing rats is, as yet, not explained. The possibility certainly exists that an inhibitory substance possessed by their normal mothers has been transferred to the young either through the placenta or through the milk. Since precautions were taken to exclude the possibility of accidental infections of the mothers, we believe that these substances are not the specific immune bodies which Miller (7-8) has shown to be transmitted from immune mothers to young. On the other hand, the intestine of the very young rats may lack the enzymes required to free the embryos by digesting the hard and thick envelope of the administered onchospheres. The fact that some development goes on in the older nurslings, even though the cysts generally die or attain only small size, seems to favor the view that the mother rat does transfer an inhibiting substance of perhaps a non-specific character to the young. Such transfer is by no means proved, however, by the data thus far obtained.

SUMMARY

An age resistance is described for the rat against *Cysticercus fasciolaris*. Rats from 25 to 60 days old are more susceptible to this parasite

than are younger or older rats. Newly born rats can seldom be infected at all, and older nurslings yield chiefly dead cysts. Rats infected when four months old or more likewise seldom yield mature cysts, although dead cysts are usually observed in these animals.

BIBLIOGRAPHY

1. OTTO, G. F. 1935 Human infestation with the dwarf tapeworm, *Hymenolepis nana*, in the Southern United States. J. Parasitol. 21: 443.
2. SHORB, D. A. 1933 Host-parasite relations of *Hymenolepis fraterna* in the rat and mouse. Am. J. Hyg. 18: 74.
HUNNINEN, A. V. 1935 Studies on life history and host-parasite relations of *Hymenolepis fraterna* (*H. nana*, var. *fraterna* Stiles) in white mice. Am. J. Hyg. 22: 414.
3. ACKERT, J. E. AND REID, W. M. 1937 Age resistance of chickens to the cestode *Railletina cesticillus* (Molin). J. Parasitol. 23: 558.
4. THOMAS, L. J. 1940 On the natural elimination of bird tapeworms. Anat. Rec. 78 Suppl.: 104.
5. CURTIS, M. R., DUNNING, W. F. AND BULLOCK, F. D. 1933 Genetic factors and etiology of malignant tumors. Am. J. Cancer 17: 894.
6. MILLER, H. M. AND GARDINER, M. L. 1932 Passive immunity to infection with a metazoan parasite, *Cysticercus fasciolaris*, in the albino rat. J. Prev. Med. 6: 479.
7. MILLER, H. M. 1932 Transmission to offspring of immunity against infection with a metazoan (cestode) parasite. Proc. Soc. Exper. Biol. & Med. 29: 1124.
8. MILLER, H. M. 1935 Transmission to offspring of immunity against infection with a metazoan (cestode) parasite. Am. J. Hyg. 21: 456.

STUDIES ON SOME GORDIACEA OF NORTH AND SOUTH AMERICA*

JOSÉ CANDIDO M. CARVALHO

Few studies on American GORDIACEA have been made since the works of Montgomery (1898-1907) and May (1919). The present paper deals with some North and South American species studied in the light of recent revisions of the group especially by Heinze (1934-1937).

HISTORICAL

Linnaeus (1766) erected the genus *Gordius* for certain species now included in the class GORDIACEA and the nematode families FILARIIDAE and MERMITHIDAE. Gmelin (1788) divided the genus *Gordius* into *Gordius* and *Filaria*. Dujardin (1842) named the genus *Mermis* and considered it belonged among the nematodes. Creplin (1847) separated the genus *Chordodes* from *Gordius*. Villot in his monograph (1874) referred all 34 of the then known species of GORDIACEA to *Gordius*, but *Chordodes* was accepted and clearly defined by Janda (1893). Camerano (1897) added the genera *Paragordius* and *Parachordodes*. Camerano's (1915) revision listed 113 species which he considered valid. Most of the taxonomic studies of GORDIACEA in America have been made by Montgomery (1898-1907). May (1919) described the families GORDIIDAE and CHORDODIDAE and studied the life cycles of *Gordius robustus* Leidy and *Paragordius varius* (Leidy). G. W. Müller (1927) split the genus *Parachordodes* into *Parachordodes* and *Gordionus*. Heinze (1933-1937) erected the genera *Beatogordius* (1934), *Paragordionus* (1935), *Chordodiolus* (1935) and *Euchordodes* (1937), and four sub-families (1935) of CHORDODIDAE. Heinze (1937) also developed new techniques in preparing specimens (especially the cuticula) for study. Dorier (1930) showed that larvae of GORDIACEA may encyst on vegetation and infect their host by mouth.

Many species of GORDIACEA have been named without sufficient description to distinguish them. Identification must be based on details of the extremities and especially on the minute structure of the cuticula: its areolae, pore canals, furrows, tubercles, bristles.

Received for publication, July 23, 1941.

* Studies from the Zoological Laboratories, the University of Nebraska, No. 209.

These studies were made under the direction of Dr. H. W. Manter whom the author wishes to thank for the loan of specimens and for many valuable suggestions. Specimens were also made available by Raymond Roberts and M. J. Harbaugh of the University of Nebraska.

METHODS AND MATERIALS

Adult GORDIACEA are collected from fresh-water habitats. Immature to adult specimens can be secured by dissection of the insect hosts (usually grasshoppers, crickets, or cockroaches). Individuals in a knot of more than 50 specimens of GORDIACEA, probably *Paragordius varius* (Leidy) found free-living in Kansas were still immature, demonstrating (as claimed by Camerano and Villot) that free-living forms are not necessarily mature.

For study of the extremities, a worm should be cut somewhat anterior to the cloacal aperture and posterior to the dark ring. For the study of the cuticula, cut small pieces from the middle third of the body. It is also desirable to secure an entire circumferential piece which, after some time in lacto-phenol solution, can be released from the underlying musculature. The cuticular structure often shows differentiation near the median lines. The technique of Müller (1927) and Heinze (1937) was used in making preparations of the cuticula. Study should be made of pieces in alcohol-diluted glycerin; lactophenol solution; water; Canada balsam and allowed to dry superficially. When possible histological sections of the cuticula are made since the surface examination alone is sometimes not enough, especially in the subfamily CHORDODINAE where minute details of areolae are important in specific diagnosis.

At least a combination of the cuticula and the posterior extremity must be used for specific characters. The shape of the posterior end alone is a weak character subject to many variations as verified by the author in females of *P. varius* and also in the postanal crescent of *G. robustus*. Drawings of the cuticula are almost necessary with descriptions. The author found that properly prepared pieces of cuticula lend themselves well to photomicrographs. Some indication was noted that polarized light might reveal added differentiation in the cuticula of some species.

TWO NEW RECORDS FROM MAN

Among the specimens studied was one adult female of *Paragordius varius* recovered, several years ago, from a child patient by Dr. G. R. Underwood of Lincoln, Nebraska. The specimen was passed in the feces in a living condition.

The second case is represented by an adult female of a *Paragordius* species (described below) given to the author by Dr. Ruy Gomes de Moraes, Professor of Parasitology, National Medical School, Rio de Janeiro, Brazil. It was expelled alive from the urethra with severe pains suffered by the child host. The girl patient had frequently complained of pains in the inguinal region. The antecedents of this strange infection are not known, and it is the first, so far as the author can determine, reported from the urinary passages.

TAXONOMY

Today, the class GORDIACEA (= NEMATOMORPHA) is subdivided as follows:

Order NECTONEMATOIDEA Rauther, 1930 (all marine)

Order GORDIOIDEA Rauther, 1930

Family CHORDODIDAE May, 1919

Subfamily CHORDODINAE Heinze, 1935

Genus *Chordodes* Creplin, 1847

“ *Euchordodes* Heinze, 1937

Subfamily CHORDODIOLINAE Heinze, 1935

Genus *Chordodiolus* Heinze, 1935

Subfamily PARAGORDIINAE Heinze, 1935

Genus *Paragordius* Camerano, 1897

Subfamily PARACHORDODINAE Heinze, 1935

Genus *Parachordodes* Camerano, 1897

“ *Gordionus* Müller, 1927

“ *Beatogordius* Heinze, 1934

“ *Paragordionus* Heinze, 1935

Family GORDIIDAE May, 1919

Genus *Gordius* Linnaeus, 1766

Müller (1927) and Heinze (1934, 1935, 1937) have split the genus *Parachordodes* into a number of genera largely on the basis of the cuticula structure. The author believes that the genus *Chordodes* can well be divided into three genera based on the criteria used by Müller and Heinze for the genus *Parachordodes*. The following revision is proposed:

Genus *Chordodes* (Creplin, 1847) emend.

Posterior extremity of male not bilobed, with only comparatively shallow groove on ventral surface extending from cloaca to distal end. Posterior end of female usually swollen, entire, with terminal cloacal aperture. Cuticula usually with two or more types of papillar areolae, very prominent, variably grouped, the highest ones often bearing crown or tuft of transparent, long and slender filaments. Above areolae, or among them, scattered tubercles, hyalin processes and other structures are present. In most species, at least in female, there is median ventral furrow in which papillar areolae are more numerous and very conspicuous.

The genus comprises usually large species with geographical distribution confined to tropical or equatorial regions.

Type species: *Chordodes pilosus* Möbius, 1855.

Genus *Neochordodes* n. g.

(*Chordodes* (Creplin, 1847) pro parte)

(Fig. 11)

Tropical CHORDODINAE of unusually large size; anterior extremity slender, cylindrical; head attenuated or not, with obtusely truncated or rounded tip. Body cylindrical; posterior end narrower than immediately preceding portion, slightly flattened dorso-ventrally, rounded in female. In most species male provided with shallow median, ventral cloacal groove. Cuticula with single type of areolae, usually ovoid or rounded-polygonal in outline, very low in comparison with those of *Chor-*

dodes. Interareolar furrows with or without hyalin processes, bristles, or other structures.

Type species: Neochordodes talensis (Camerano, 1897) n. comb.

Other species: N. annadalei (Camerano, 1908) n. comb.; *N. molukkanus* (Römer, 1896) n. comb.; *N. weberi* (Villot, 1891) n. comb.; *N. moraisi* n. sp. (described below).

This genus seems well characterized by the shape of the posterior end of the male and by the single type of areolae. It might be mistaken for *Gordionus* which also has a single type of areolae, but it differs by the unforked posterior end of the male as well as by the lack of interareolar pore canals or rows of bristles or tubercles which are present in *Gordionus*. *Neochordodes* has only a few, scattered hyalin processes and sparse hairs or bristles. Differences from other genera are well marked.

Genus *Pseudochordodes* n. g.

(*Chordodes* (Creplin, 1847) pro parte)

Tropical CHORDODINAE of medium size. Usual shape is like genus *Chordodes*; anterior end pointed; posterior, median, ventral groove of male very weak, posterior to cloacal aperture which is usually no more than 0.5 mm from distal end. Cuticula with two types of very characteristic areolae: (1) usually polygonal areolae, mostly 5- or 6-sided, higher and darker than second type, in pairs or groups of three, rarely four, sometimes isolated. Most areolae bear pore canal with sessile bristle in narrow furrow which separates them; (2) low areolae, light in color, variable in shape and occupying greater part of cuticula. Interareolar furrows free of tubercles, bristles or other structures.

Type species: Pseudochordodes pardalis (Camerano, 1893) n. comb. (Fig. 12).

Other species: P. bedriagae (Camerano, 1896) n. comb.; *P. dugesi* (Camerano, 1898) n. comb.; *P. manteri* n. sp. (described below), and (with some question) *P. gestri* (Camerano, 1904) n. comb.

This genus differs from *Chordodes* by the presence of pore canals between the large areolae; from *Paragordionus* (which has the same type of areolae) by the unforked posterior end of the male and by the absence of interareolar structures; from *Euchordodes* by the absence of stout interareolar bristles. From other genera differences are well marked.

The author is satisfied that the following synonyms are justified. *Paragordius flavescens* Linstow, 1906 and *P. diversolobatus* Heinze, 1935 (Fig. 10) are synonyms of *P. varius*. Variation of the posterior end of the female of this species is common and unless a combination of this character is made with the cuticular structure no specific diagnosis could be surely established. The drawings of the cuticula presented by Heinze and von Linstow correspond exactly with all specimens of *P. varius* studied by the author. The bright halo mentioned and figured by von Linstow is merely a question of microscope adjustment (focus). Montgomery's drawings were evidently made under small magnification which probably led the German authors to regard their specimens as different species. The species described by Camerano as *Gordius danielis* in 1894 is considered by the author to be synonym of *Gordius paranensis*

Camerano, 1892. *Gordius guatemalensis* v. Linstow, 1902, *Gordius willeyi* Camerano, 1899 and *Gordius californicus* Camerano, 1915 should be considered as synonyms of *Gordius robustus* Leidy, 1850 since the slight variations of the shape of the lobes and crescent do not seem to be enough to separate them as different species. In the opinion of the author, *Gordius villoti* Rosa, 1882 is not a tenable species and most specimens referred to it in Europe are *G. setiger* Schneider, 1866 while the American specimens are probably *G. robustus* Leidy, 1850. The redescription of the latter species given by May (1919) agrees with specimens studied by the author.

Pseudochordodes manteri n. sp.

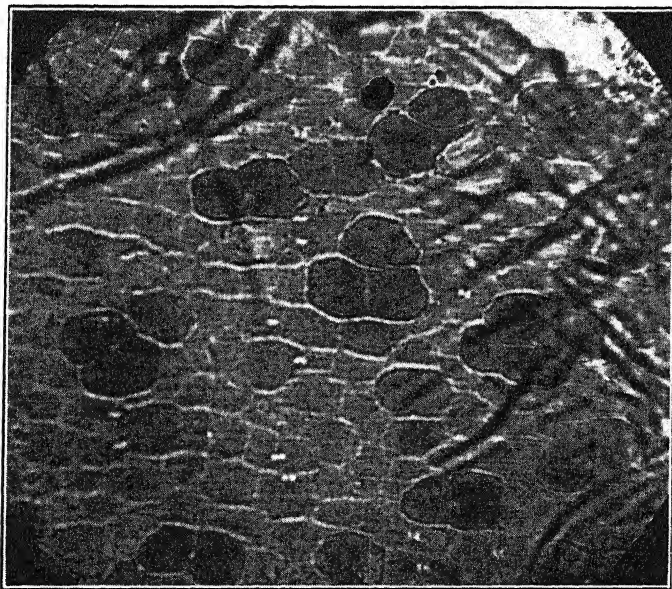
(Text-Fig. 1)

Dimensions: Length female, 160 mm; diameter near anterior end 0.354 mm; at midbody 0.9 mm, before swelling of posterior end 0.446 mm.

Form: Posterior end slightly pointed; sides of head almost parallel; mouth terminal. Dark ring, dorsal and ventral lines not visible. Posterior end with swelling near distal end, decreasing abruptly to more or less pointed posterior end. Cloaca terminal.

Color: Specimen (in alcohol) is dark; examined under light it has brownish color uniform over entire body.

Cuticula: With two types of areolae: (1) Large paired areolae, sometimes 3 or 4 together, scattered among second type and usually darker in color; rounded-elon-



TEXT-FIG. 1. Photomicrograph of the cuticula of *Pseudochordodes manteri* in lactophenol solution showing two types of areolae, interareolar furrows, pore canals and scattered paired tubercles (light spots) above some of the small areolae. The areolae are extended transversely. The dark fibrous shadows at right and upper left are remnants of body musculature (circa $\times 450$).

gated or coarsely polygonal with rounded corners. These areolae higher than other type and larger diameter transverse to long axis of body. As characteristic for genus, they include pore canal between them in line that separates a pair or in center of 3 or 4 areolae. (2) Small, polygonal areolae usually four-cornered, sometimes triangular or pentagonal, extending crosswise to long axis of body and occupying most of cuticula, separated by narrow furrows showing no special structures. This type of areola is only type occurring in regions of dorsal and ventral lines where areolae are strongly polygonal, very irregular in size and form, extended longitudinally, and where interareolar furrows are not so visible as on sides of body. In region of ventral line, areolae form band about 0.15 mm wide. Characteristic for this species is presence of scattered paired tubercles above some of small areolae.

Areolae in general easily seen in lactophenol and in Canada balsam. External structure and paired tubercles are better visible in dried pieces of cuticula.

Locality: War Bonnet Canon, Nebraska, U. S. A. Specimen collected in freshwater.

Type: One female, deposited in the U. S. Nat. Mus., No. 36810.

This species seems to be similar to *Pseudochordodes pardalis* (Camerano, 1893) from Madagascar. It differs as follows:

- | <i>P. manteri</i> (Fig. 3) | <i>P. pardalis</i> (Fig. 12) |
|---------------------------------------------------------------------------------------|---------------------------------------------------------|
| 1. Large areolae without tubercles. | 1. Large areolae with tubercles. |
| 2. Small areolae bear one tubercle which often approximates another to appear paired. | 2. Small areolae very sinuous, without tubercles. |
| 3. Dorsal and ventral lines with small areolae only. | 3. Dorsal and ventral lines with both types of areolae. |

In addition, in *P. manteri* the small areolae and their furrows run transversely to the long axis of the body (Text-Fig. 2); the color is uniform, without spots; the shapes of the extremities are different. From *P. bedriagae* (Camerano, 1896) and *P. dugesi* (Camerano, 1898) differences are marked. The species is named in honor of Dr. H. W. Manter of the University of Nebraska.

Paragordius esavianus n. sp.

(Figs. 1-3)

Dimensions: Length female 211 mm, largest diameter 1 mm, diameter at dark ring about 0.39 mm, at level of trifurcation of lobes 0.55 mm.

Form: About 7 mm from posterior end (just posterior to atrium) abrupt narrowing of body from 0.693 to 0.492 mm. Anterior end pointed; dark ring distinctly visible so far anterior that with naked eye it seems at anterior end. With magnification, tip of head yellowish. Dorsal and ventral lines faintly visible. In region of dorsal and ventral lines and on sides of body are longitudinal, somewhat indistinct cuticular ridges visible in pieces of cuticula after releasing musculature. Posterior end (of female) trilobed, each lobe about 1.555 mm long; dorsal lobe slender, diameter at base 0.215 mm, in the middle 0.169 mm, near the tip 0.200 mm. This dorsal lobe has characteristic shape, narrower in middle, constriction near base. Lateral lobes 2 or 3 times wider than dorsal, diameter at base 0.539 mm, in middle 0.400 mm, near tip 0.231 mm, somewhat pointed and bearing internally pronounced excavation. They are very little divergent, practically in contact ventrally throughout entire length. On median and outer surfaces of lobes, in addition to spine-like cones of

cuticula, there are long, filiform hairs visible especially at the margins of the excavation.

Color: Brownish yellow with very marked dark ring. Extremities do not differ from body in color.

Cuticula (Fig. 3): Entire surface of body evenly covered by numerous spine-like conical tubercles especially well seen in dried pieces of skin or in pieces in water under coverglass. In other liquids not well seen. When pieces of skin are superficially dried after being in diluted glycerin or lactophenol, cuticula shows formation of false areolae due to accumulation of liquid at bases of tubercles so that regular pseudo-areolar temporary formations are seen. In histological sections tubercles appear pointed with slightly rounded apex. They are straight and do not bear any refractive structure or hairs. Pore-canals and inter-tubercular bristles are apparently absent.

Locality: Cachoeira do Itapemirim, Estado do Espirito Santo, Brazil.

Host: This specimen, collected by Dr. Ruy Gomes de Morais, School of Medicine, Rio de Janeiro, Brazil, was expelled by girl through urethra, and seems to be first record of pseudo-parasitism by GORDIACEA of human urinary tract.

Type: One female, in collection of National Museum, Rio de Janeiro, Brazil.

This species differs markedly from others in the genus by the shape of the posterior end of the female and by the spine-like conical tubercles covering the cuticula. Very characteristic is the abrupt narrowing of the body in the region of the atrium not far from the posterior end. The species is named for E.S.A.V. (Escola Superior de Agricultura e Veterinaria, Viçosa, Minas Gerais, Brazil).

Neochordodes moraisi n. sp.

(Text-Fig. 2)

Dimensions: Length female 207 mm; diameter anteriorly 0.246 mm, in middle of body 2 mm, at narrow portion of posterior end of body 0.280 mm, short distance anterior to narrow portion of posterior end, 1.078 mm.

Form: Anterior end strongly attenuated, tip of head rounded. Body increases in thickness gradually toward middle and decreases again toward posterior end. Posterior end club-shaped. Cloacal aperture terminal and situated in middle of truncated distal end surrounded by yellowish area. Tip of head free of areolae but with small granulations. Dorsal and ventral lines and dark ring apparently absent.

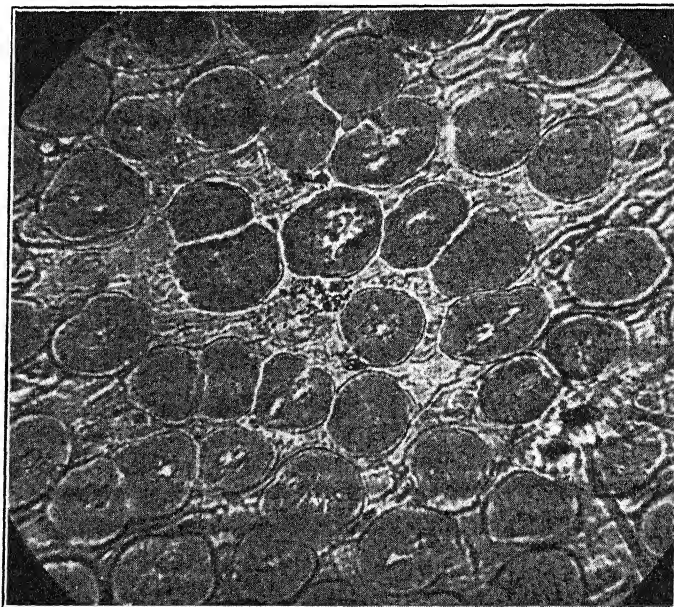
Color: Yellowish brown, body covered throughout by very irregular brown spots which, joining here and there, give rise to larger spots. Anterior end slightly lighter and posterior end yellowish white around cloacal aperture.

Cuticula (Text-Fig. 2): With one type of areolae usually rounded or ovoid, often with indentation on one side, with two, rarely three or more conical elevations more refractive. These usually paired above areolae. They may be found widely separated on areolar surface and sometimes near margins of two paired areolae giving false idea of pore-canals. Indentations of areolae usually well marked and reaching base of elevations. Greater diameter of areolae is transverse to long axis of body. Usually, areolae are isolated or in pairs but sometimes three or four together. Inter-areolar spaces wide, showing several furrows with some superficial ridge-like elevations. Scattered hyalin processes and fine tubercles in furrows. With low magnification, areolae appear arranged more or less in straight lines crossing long axis of body, and elevations as bright granules in pairs over areolae. In sections of cuticula, areolae show same height and elevations conical in shape with rather pointed tips. Here and there occur hyalin processes nearly same height as areolae. Appearance of cuticula in different liquids is rather constant. Areolae very distinct in diluted glycerine and in superficially dried pieces of cuticula.

Locality: E.S.A.V., Viçosa, Minas Gerais, Brazil.

Host: Collected by author from cockroach, *Blatta orientalis* Linn., in very poor condition, evidently severely affected by infection. (The striking and unusual development of the abdomen of the cockroach had attracted attention. After removal of the worm, one could scarcely believe that such a large worm had come from inside a cockroach.)

Type: One female, in collection of National Museum, Rio de Janeiro, Brazil.



TEXT-FIG. 2. Photomicrograph of the cuticula of *Neochordodes moraisi* in lactophenol solution showing the single type of areolae, and wide interareolar spaces with ridge-like elevations. Some of the areolae show two or three of conical elevations (circa $\times 450$).

Areolae of one type with conical, paired or sometimes one or three prolongations do not occur in any other species of the genus.

This species is named in honor of Dr. Ruy Gomes de Morais.

SUMMARY

1. Two new cases of human infection with GORDIACEA are reported. One of these involved infection of the urinary passages.
2. The genus *Chordodes* is split into the genera *Chordodes*, *Neochordodes* and *Pseudochordodes*. The latter two are new.
3. *Gordius danielis* Camerano, 1894 is considered a synonym of *G. paranensis* Camerano, 1892. *G. guatemalensis* v. Linstow, 1902, *G. willeyi* Camerano, 1899 and *G. californicus* Camerano, 1915 are considered synonyms of *G. robustus* Leidy, 1850. *G. villoti* Rosa, 1882 is considered a synonym of *G. setiger* Schneider, 1866.

4. *Paragordius esavianus*, *Pseudochordodes manteri* and *Neochordodes moraisi* are described as new species.

BIBLIOGRAPHY

- CAMERANO, L. 1897 Monografia dei gordii. Mem. R. Accad. Sc. Torino 47: 339-419.
 ——— 1915 Revisione dei gordii. Mem. R. Accad. Sc. Torino 66: 1-66.
 DORIER, A. 1930 Recherches biologiques et systématiques sur les gordiacés. Trav. Lab. Hydrobiol. et Piscicult. Univ. Grenoble, 183 pp.
 DUJARDIN, F. 1842 Mémoire sur la structure anatomique des Gordius et d'un helminthe le Mermis, qu'on a confondu avec eux. Ann. Sc. Nat. Zool. 18: 129-151.
 HEINZE, K. 1934 Zur Systematik der Gordiiden. Zool. Anz. 106: 189-192.
 ——— 1935 Über das Genus *Parachordodes* Camerano, 1897 nebst allgemeinen Angaben über die Familie Chordodidae. Z. Parasitenk. 7: 657-678.
 ——— 1937 Die Saitenwürmer (Gordioidea) Deutschlands. Eine Systematisch-faunistische Studie über Insectenparasiten aus der Gruppe der Nematomorpha. Z. Parasitenk. 9: 263-344.
 JANDA, J. 1893 Beiträge zur Systematik der Gordiiden. Zool. Jahrb., Syst. 7: 595-612.
 MAY, H. G. 1919 Contribution to the life histories of *Gordius robustus* Leidy and *Paragordius varius* (Leidy). Illinois Biol. Monogr. 5(2): 1-118.
 MONTGOMERY, T. H., JR. 1898a The Gordiacea of certain American collections, with particular reference to the North American fauna. Bull. Mus. Comp. Zool. Harvard Coll. 32: 23-59.
 ——— 1898b The Gordiacea of certain American collections with particular reference to the North American fauna. II. Proc. Calif. Acad. Sc. 1(3): 333-344.
 MÜLLER, G. W. 1927 Über Gordiaceen. Z. Morphol. u. Oekol. Tiere 7: 134-218.
 ROMER, F. 1896 Beiträge zur Systematik der Gordiiden. Abh. Senckenberg. Nat. Ges. 23: 247-295.
 SCHIACCHITANO, I. 1937 Nuove Gordii del Congo Belge. Rev. Zool. et Botan. Africaines 30: 141-151.
 VILLOT, A. 1874 Monographie des dragonneaux (genre *Gordius*, Dujardin). Arch. Zool. Expér. et Gén. 3: 181-238.
 ——— 1886 Révision des gordiens. Ann. Sc. Nat., Zool. 1: 271-318.

EXPLANATION OF PLATE I, p. 222

Figs. 1-8 were drawn with the aid of a camera lucida.

- FIG. 1. Posterior end of *Paragordius esavianus* (female), ventral view.
 FIG. 2. Posterior end of *P. esavianus* (female), dorsal view.
 FIG. 3. Spine-like conical tubercles of *P. esavianus* (female).
 FIG. 4. Posterior end of *P. varius* (female), dorsal view.
 FIG. 5. Group of areolae of *P. varius*, showing bright areas due to microscope adjustment (focus).
 FIG. 6. Posterior end of *P. varius* (male), compressed, ventral view.
 FIGS. 7-9. Different groups of areolae of *P. varius*.
 FIG. 10. Areolae of *P. diversolobatus* (after Heinze, 1935). Showing similarity to areolae of *P. varius*.
 FIG. 11. Cuticula of *Neochordodes talensis* (after Camerano, 1897). Showing single type of areolae and granulations in interareolar furrows.
 FIG. 12. Cuticula of *Pseudochordodes pardalis* (after Camerano, 1893). Showing large areolae with tubercles, and small areolae with sinuous irregular outlines.

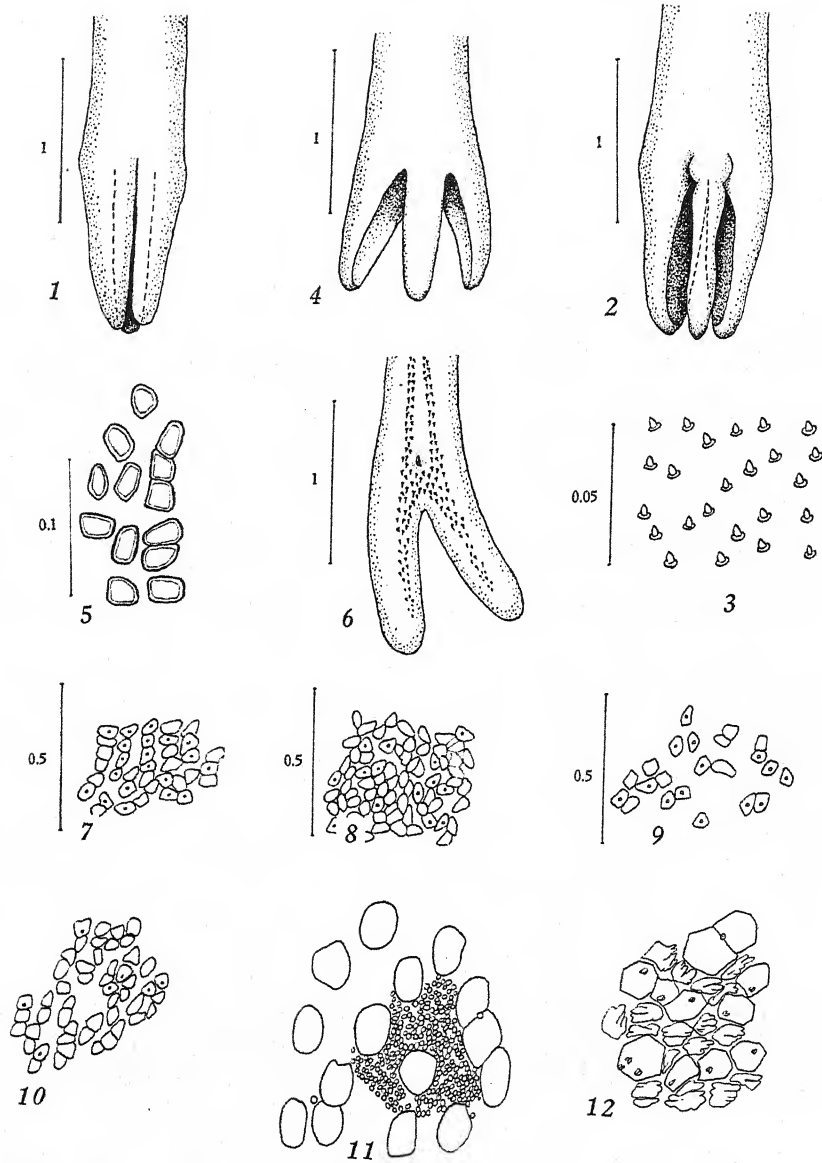


PLATE I

TRICHINELLA SPIRALIS. II. INCIDENCE OF INFECTION IN HOGS AND RATS IN THE NEW ORLEANS AREA

CHARLES E. PERES*

Department of Tropical Medicine, Tulane University of Louisiana, New Orleans, La.

Hinman (1) and Sawitz (2) through examination of autopsies have demonstrated that human infection with *Trichinella spiralis* occurs in the New Orleans area. Studying two square inches of diaphragm from each of 200 autopsies, Hinman found an incidence of 3.5 per cent. Sawitz, using larger samples and examining 400 autopsies, determined the incidence to be 6.0 per cent. In an earlier study Sawitz (3) detected an incidence of 5.0 per cent in 200 living persons by means of the intradermal test. In addition to man, rats, dogs and cats from this area have been examined. An incidence of 0.5 per cent was found in 200 rats (1), of 1.3 per cent in 300 dogs, and 10 per cent in 90 cats (2).

The present study was undertaken to determine the sources of human infection and to complete the studies on the incidence of *Trichinella spiralis* infection in its principal hosts in this area. This entailed the examination for *Trichinella* of both hogs and rats, and in the case of the latter, especially those rats which had had opportunity for association with hogs or pork.

SOURCE OF MATERIAL

Pork consumed in New Orleans comes from hogs that are raised in the South (Louisiana, Mississippi and Alabama), hogs that are raised on the outskirts of the city, and those that are raised in the Midwest. Southern hogs, including those raised near the city, are shipped in alive and are slaughtered locally. Midwestern hogs are usually received already slaughtered and dressed, the principal product being loins. Since no figures on the actual consumption of pork are available, the figures presented in Table 1 have been computed from municipal tax receipts.

TABLE 1.—Hogs consumed in New Orleans as indicated by municipal tax receipts

	1934	1935	1936	1937	Total
Southern hogs	21,816	20,156	30,708	36,285	108,965
Midwestern hogs . . .	90,552	58,494	84,850	78,391	312,287
Total	112,368	78,650	115,558	114,676	421,272

These figures based upon tax receipts are not complete, because there

Received for publication, July 28, 1941.

* Aided by a grant from the Committee on Scientific Research of the American Medical Association.

The writer wishes to express his appreciation to Dr. E. C. Faust for courtesies extended and helpful criticism.

are several concerns which sell pork in New Orleans, but do not operate branch houses, and do not pay the tax. The number of these hogs may be estimated at 25,000 annually. Combining these figures with those presented in Table 1 it is seen that the pork supply of New Orleans consists of approximately four-fifths Midwestern hogs and one-fifth Southern hogs.

There is a difference in the food supply of these hogs. The hogs raised on the outskirts of the city are fed mostly upon garbage; those from other parts of the South are allowed to roam in the fields and uproot peanuts where they are present; and those from the Midwest are fed largely upon grain. It seems reasonable to assume, however, that all hogs receive some garbage, even if it is only the unused material from the family table. In the present study hogs were examined from the sources mentioned above.

During this study rats also were examined, special emphasis being placed on those frequenting local hog farms, the two local abattoirs, and two local rendering plants.

METHODS OF EXAMINATION OF MATERIAL

Pork samples of Southern hogs were obtained in the abattoirs immediately after the animals had been slaughtered. Large pieces of diaphragm were cut from the hogs. Midwestern pork samples were obtained from branch houses of firms operating branches in the city and from butchers handling pork of packers not operating New Orleans branches. All samples of Midwestern pork were cut from fresh, unfrozen loins.

The pork samples were prepared for examination by the artificial digestion technic. A solution of 1.0 per cent pepsin in 0.6 per cent hydrochloric acid was allowed to act upon 50-gram samples of the ground meat at a temperature of 38° C for 15 to 20 hours. During this time occasional stirring was done. The resulting material was strained through two layers of cheesecloth into a large funnel which was equipped with a short piece of rubber tubing having a screw clamp at the delivery end. The material was allowed to settle for 2 to 3 hours, during which time the liquid in the upper part of the funnel was lightly stirred once or twice. After this sedimentation period, about 30 ml. of the bottom material were drawn off into a Petri dish and examined. In addition, approximately 250 samples were examined by the compressor technic.

The rats studied were caught in guillotine-type traps on local hog farms, the two local abattoirs, and two local rendering plants. The diaphragms of these rats were removed and immersed in 50 per cent glycerin solution. After 24 hours they were transferred to undiluted glycerin and, after another 24 hours, compressed between two slides and examined.

RESULTS OF EXAMINATIONS

Five hundred sixteen Southern hogs were examined and found negative for *Trichinella*. Seven hundred ninety-two loins representing at least 399 Midwestern hogs were examined and also found negative. Fifty samples of pork sausage bought from butchers and probably made up of both Southern and Midwestern pork, were examined by artificial digestion with negative results. Thus examination of 915 hogs and 50 samples of pork sausage consumed in New Orleans revealed no presence of *Trichinella*.

Of 467 rats examined, two were found infected with *Trichinella*, an incidence of 0.4 per cent. One of the positive rats was trapped on one of the hog farms, the other in a rendering plant located near several hog farms. In none of 280 rats from the abattoirs was *Trichinella* infection detected.

DISCUSSION

Although the studies of Hinman and Sawitz indicate an incidence of 5 to 6 per cent *Trichinella* infection in the human population of the New Orleans area, an examination of pork as the source of infection has revealed no positives among 915 hogs sampled. Furthermore, 50 samples of pork sausage were also negative.

The failure to find any positives in 516 Southern hogs indicates that the percentage of infection in the hog population of this area is low. Calculated from the binomial distribution and Fisher's fiducial probability theory, the probability is only 0.025 that the percentage of infection in these hogs exceeds 0.73 per cent. A similar low incidence in the South was determined in 1881, by Deverson (4), who detected only 0.4 per cent positives among 5400 hogs and, in 1883, by Detmers (5) who found no positives in 4146 Southern-raised hogs.

The significance of the failure to find any infected hogs in 399 Midwestern hogs may be expressed statistically as a probability of only 0.025 that the percentage of infection in that group exceeds 0.95 per cent. After an examination of thousands of samples Hall (6) reported incidences of 1.5 and 4.8 per cent respectively for grain-fed and garbage-fed Midwestern hogs. Schwartz (7) reported incidences of 1.0 and 5.0 per cent for similar groups. Hood and Olson (8) found a 0.4 per cent incidence in 500 grain-fed Midwestern hogs.

The 0.4 per cent *Trichinella* infection in 467 rats examined approximates Hinman's results (1) of 0.5 per cent in 200 rats taken at random in New Orleans. These comparable figures in rats indicate no appreciable difference in *Trichinella* incidence in rats associated with hogs as compared with rats living in the metropolitan area of New Orleans.

Of special interest is the absence of positives in 280 rats taken from the abattoirs. Many of these rats probably had opportunity to feed

upon stored pork, and if trichinous pork were occasionally stored in the abattoirs, the incidence of *Trichinella* in these abattoir-rats should be greater than in rats not frequenting places of pork storage. Thus the absence of infection in these 280 rats is further evidence of the low incidence of *Trichinella* infection in Southern hogs.

While the actual source of the 5 to 6 per cent incidence in man has not been detected in this study, it may be assumed that this infection rate is maintained by an occasional infected hog which would be found only by examination of much larger numbers of hogs.

The incidence of *Trichinella* infection in its main hosts in the New Orleans area may be summarized from the present data and those of Sawitz (2) by using his epidemiological diagram:

		dogs 1.3%
man 5-6%	hogs 0-1%	rats 0.4%
		cats 10%

SUMMARY

During 1938 and 1939 the diaphragms of 516 Southern hogs and the loins of 399 Midwestern hogs were examined for *Trichinella* infection by the artificial digestion technic. None was found infected. Likewise, none of 50 samples of sausage from local butchers revealed infection. Two positives, or 0.4 per cent, were detected in 467 rats trapped in this area. These low incidences agree with the low infection rate in man in the New Orleans area, as contrasted with other areas of the United States.

BIBLIOGRAPHY

1. HINMAN, E. H. 1936 Trichiniasis in Louisiana. New Orleans Med. and Surg. J. 88: 445-448.
2. SAWITZ, W. 1939 *Trichinella spiralis*. I. Incidence of infection in man, dogs and cats in the New Orleans area as determined in post-mortem examinations. Arch. Path. 28: 11-21.
3. SAWITZ, W. 1937 Are post-mortem statistics on trichinosis valid for the living population? Am. J. Pub. Health 27: 1023-1024.
4. DEVERSON, 1881. Cited by Hall, M. C. 1937 Studies on trichinosis. IV. The role of the garbage-fed hog in the production of human trichinosis. Pub. Health Rep., U. S. Pub. Health Service 52: 873-886.
5. DETMERS, 1883. Cited by Hall (4).
6. HALL, M. C. 1935 Ann. Rep., Chief Bur. Animal Industry, 1935, Washington, pp. 48-55.
7. SCHWARTZ, B. 1936 Ann. Rep., Chief Bur. Animal Industry, 1936, Washington, pp. 53-60.
8. HOOD, M. AND OLSON, S. W. 1939 Trichinosis in the Chicago area. Am. J. Hyg. 29: 51-56.

MESOCESTOIDES MANTERI N. SP. FROM A LYNX, WITH
NOTES ON OTHER NORTH AMERICAN SPECIES
OF MESOCESTOIDES

ASA C. CHANDLER

Biological Laboratory, Rice Institute, Houston, Texas

There is considerable difference of opinion with respect to speciation in the genus *Mesocestoides*. Most writers, including Cameron (1925), Mueller (1928, 1930) and Joyeux and Baer (1932) recognize a considerable number of species parasitic in mammals, whereas Witenberg (1934) merges them all in the species *litteratus*, of which he recognizes three "forms" of doubtful status.

I have not had an opportunity to examine specimens of *Mesocestoides* from other parts of the world, so am not in a position to pass judgment on Witenberg's conclusions with respect to the forms which he studied from Palestine and Europe, but I have collected and examined specimens from various North American mammals which in my opinion undoubtedly represent three distinct species. Specimens obtained from an opossum are readily recognizable as Mueller's species, *latus*, originally reported from a skunk in Minnesota, and later from a kitten in Minnesota and an opossum in Illinois. Specimens from a raccoon from Lufkin, Texas, and some fragments from a dog and from a raccoon from Nebraska, reported by Coatney (1936) and kindly sent to me for study by Dr. H. W. Manter, correspond closely with Mueller's *variabilis* var. *maior*, reported from a skunk in California. Some specimens from a lynx in Nebraska, also kindly supplied for study by Dr. Manter, undoubtedly constitute a new species, for which the name *Mesocestoides manteri* is proposed.

Mesocestoides latus Mueller, 1928

Four specimens were obtained from an opossum at Houston, Texas, none possessing fully ripe segments, measuring 34.5, 76, 106, and 112 cm in length, thus exceeding the greatest length previously reported for this species (50 cm) by Mueller (1930). The 76 cm specimen had about 800 proglottids. Mature segments are about 1.4 to 1.8 mm wide and 0.45 to 0.7 mm long. The segments become approximately square at about the 600th segment. The terminal segments of the longest worm, which are almost fully ripe, are 3.4 mm long and 1.6 mm wide. This worm, as pointed out by Mueller, lacks calcareous corpuscles, and becomes very clear on prepared slides as compared with other species. Another characteristic feature, not mentioned by Mueller, is the sharply defined walls of

Received for publication, August 5, 1941.

the vagina; an enlarged portion of this organ serving as a seminal receptacle, lying just anterior to the cirrus pouch, persists even in the terminal segments of the worm, and is almost as conspicuous as the cirrus pouch. Still another peculiarity is the method of formation of the egg capsule (Fig. 1, g-k). This develops from a mass of dense tissue at the posterior end of the uterus, which differentiates into a carrot-shaped organ with thick fibrous walls and a broad lumen. The egg capsule develops from the middle portion of this organ, the anterior portion having a broad lumen through which eggs reach the capsule, the posterior part contracting into a tail-like process.

Mesocestoides variabilis Mueller, 1928

Mature specimens of this worm were obtained from two raccoons from Lufkin, Texas, and some young forms still in the tetrathyridium stage were found in a third raccoon from the same locality. Two complete worms were each approximately 20 cm in length with a diameter of 1.25 to 1.5 mm. The scoleces measured 320 and 380 μ in diameter, respectively, in one case clearly demarcated from the neck, in the other not. Primordia of genital organs first appear faintly several millimeters behind the head. Mature proglottids are 550 to 840 μ broad and 280 to 400 μ long. Ripe segments are 1.8 to 2.1 mm long and 1.25 to 1.75 wide, the junction with adjoining segments being 700 to 750 μ broad.

This species is most readily distinguished from *latus* by its small scolex, by the numerous calcareous corpuscles and denser muscles which make it much less transparent, and by the method of development of the egg capsule, which first forms like a bowl around a more or less spherical mass of eggs, which may persist, although a thick-walled sphincter-like structure, as in other *Mesocestoides*, develops anterior to the mass. The fragmentary specimens from a dog and a raccoon from Nebraska are tentatively referred to this species; only semi-ripe and ripe segments are available. The former show the method of formation of the egg capsule around an egg mass which seems to be characteristic of *variabilis*. The ripe segments, 3 to 3.6 mm long and 1.3 to 1.4 broad, are much larger than my Texas specimens of *variabilis*, and resemble those of *latus*, except that they have abundant calcareous corpuscles. The difference in size may be due to method of relaxation and fixation. My specimens were obtained from a raccoon's carcass that had been frozen.

Since the specimens here reported from Texas raccoons are intermediate in size between Mueller's typical *variabilis* from *Spilogale* and *Urocyon* and his variety *maior* from *Mephitis*, it is probable that, as Mueller (1928) suspected, the distinction between these two forms is not a valid one, and the varietal name *maior* should be dropped.

Mesocestoides manteri n. sp.

(Figs. 1 a-f, 2, 3)

Description: Length 0.9 to 1.6 cm, with maximum width, in ripe segments, of 1 to 1.25 mm; total number of segments only 25 to 55. Scolex 420-490 μ in diameter and 285 to 350 μ long, set off from neck inconspicuously or not at all. Suckers in two pairs, dorsal and ventral, 175-210 μ long and 155-180 μ wide, with strong muscles, slit entire length antero-posteriorly, and directed straight dorsad and ventrad or slightly forward. Neck 315 to 500 μ broad at junction with head, almost immediately becoming as wide or wider than head. Primordia of genital organs first seen faintly about 720 μ to 1 mm from anterior end; first evidence of segmentation at edges of worm about 1 to 1.5 mm from anterior end. Segments develop rapidly, becoming fully mature at 16th to 20th segment, including those in which the primordia are only faintly present. Only a few sexually mature segments before uterus begins developing. Mature segments from 700 μ wide and 485 μ long to 900 μ wide and 175 μ long. Usually only one ripe segment present, bell shaped; junction with preceding segment only 265 to 350 μ broad.

Testes about 30 to 45 in number, closely crowded inside longitudinal excretory canals and occupying all space not occupied by other organs, 4 or 5 situated outside canals on each side; spherical or slightly oval or uneven, from 48 by 48 μ to 50 by 60 μ in size. Cirrus pouch with posterior border at about middle of segment directed antero-dorsad, 88 to 110 μ in diameter, and 88 to 140 μ long as seen from flat surface of segment; contains very much coiled sperm duct, and persists even in ripe segments.

Ovaries oval or round, 90 to 110 μ long and 70 to 110 μ broad, close together and connected by a ventral bridge, from 0 to 20 μ apart; distance between outer borders of ovaries 165 to 245 μ . Vitelline glands smaller than ovaries, ventral to them, 65 by 70 μ to 88 by 88 μ , 65 to 95 μ between them; distance between outer borders 200 to 265 μ . Vagina only moderately convoluted, passing around cirrus pouch on irregularly alternating sides, and opening just anterior to cirrus. Uterus appears first a few segments after sexual maturity is reached, bending around cirrus pouch and ending short of anterior border of segment. A few segments farther back it begins to expand and to fill with eggs, quickly forming a series of saclike bodies, one large one anterior to cirrus. Meanwhile posterior portion becomes surrounded by a mass of nuclei, and elongates and enlarges into a gourd-shaped body with a narrow lumen somewhat enlarged posteriorly, and thick striated walls, especially dense in the goose-neck region. At this stage practically no remnant of uterus visible posterior to gourd-shaped structure. Egg capsule develops from posterior portion of lumen leaving no caudal appendage (Fig. 1 a-f). In last 3 or 4 segments caudal appendage grows out anew in characteristic s-shaped form, reaching a length of 350 to 450 μ . Egg capsule slightly longer than broad, 350 by 320 μ to 395 by 350 μ . Egg mass 245 to 275 μ broad and 220 to 260 μ long, truncated posteriorly; wall of capsule thickened posteriorly where tail-like appendage forms.

Host: Lynx (*Lynx rufus*).

Habitat: Small intestine.

Locality: Nebraska, U. S. A.

Type: U. S. Nat. Mus. Helm. Coll. No. 44941.

Mesocestoides manteri is smaller than any other described forms of the genus except *M. bassarisci* MacCallum, 1921. The latter species is inadequately described, and may possibly be identical with the species here reported, but *bassarisci* is said to have the testes confined between the lateral canals, and to have the mature segments indistinctly divided from each other.

Aside from its small size and small number of segments, this species

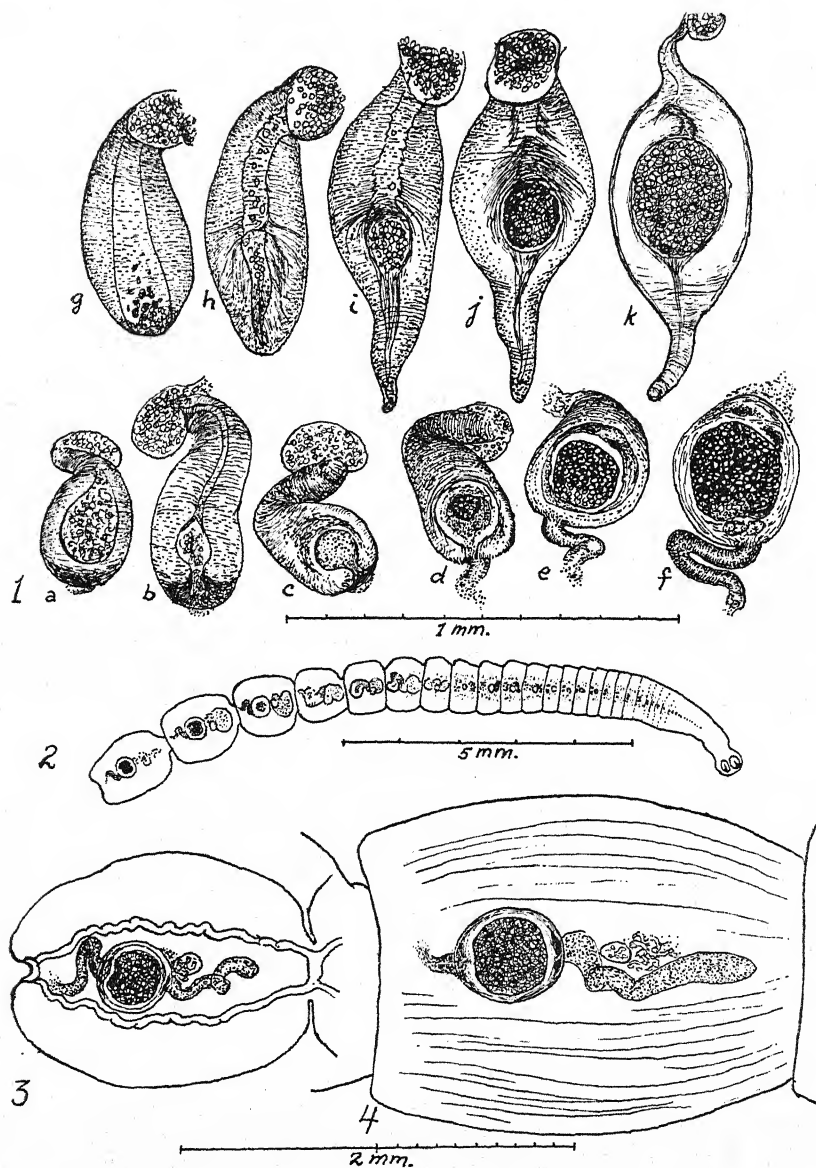


FIG. 1. Development of egg capsule from posterior part of uterus in *Mesocestoides manteri* (a-f) and *Mesocestoides latus* (g-k). Mass of eggs shown in a sometimes missing.

FIG. 2. *Mesocestoides manteri*, entire specimen.

FIG. 3. *Mesocestoides manteri*, ripe segment.

FIG. 4. *Mesocestoides variabilis* from raccoon, ripe segment, drawn to same scale as Fig. 3.

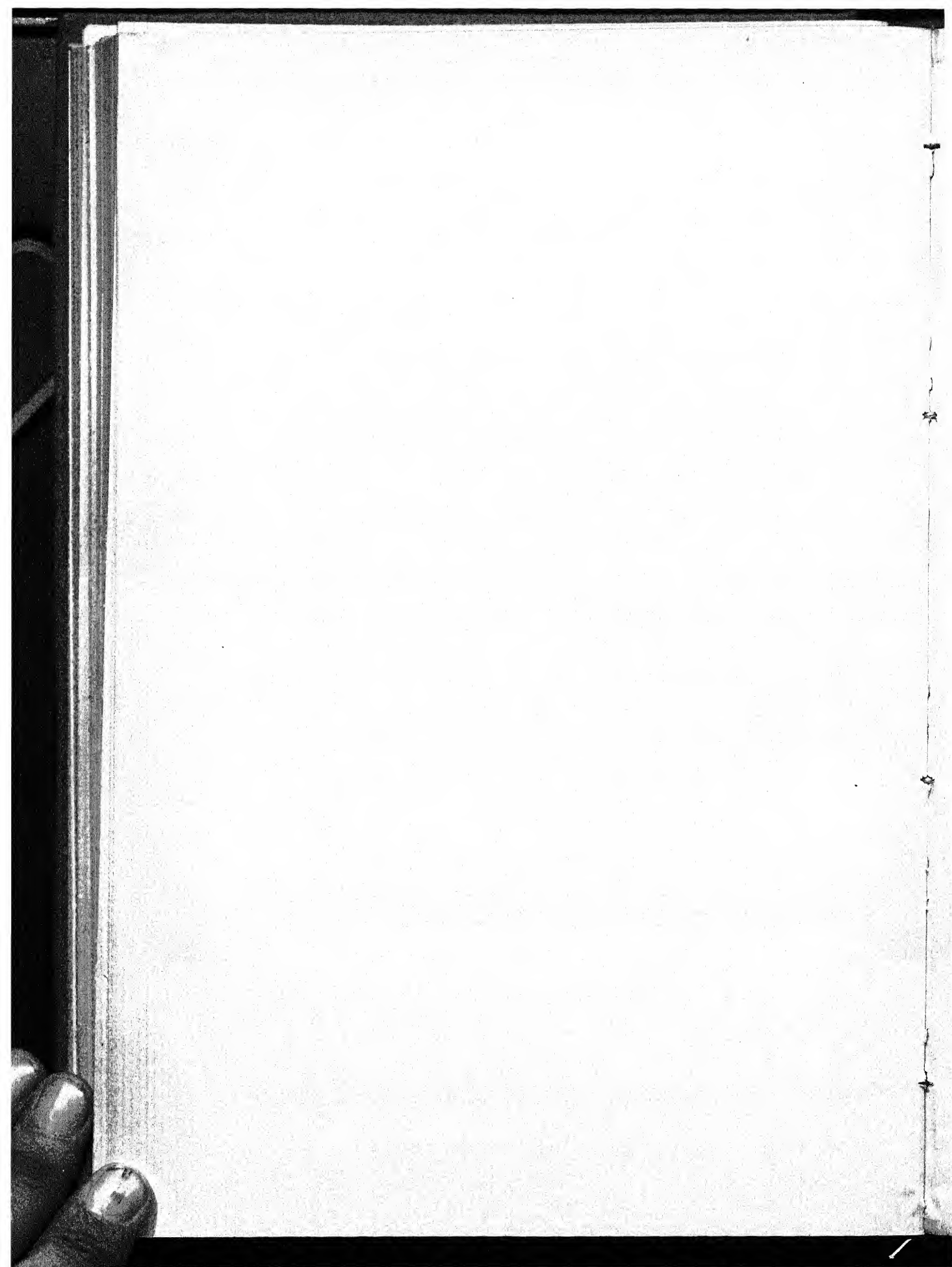
is distinguished by its relatively broad head and neck, the bell-shaped ripe segments, and the method of formation of the egg capsule and of the long, looped caudal appendage posterior to it (cf. Fig. 1 a-f and Fig. 1 g-k).

SUMMARY

A dwarf species of *Mesocestoides*, *M. manteri* n. sp., is described from a lynx in Nebraska. *M. latus* is reported from an opossum in Texas, and *M. variabilis* from a raccoon in Texas and from a raccoon and dog in Nebraska. Specific differences in method of formation of the egg capsule, and caudal appendage of the capsule, are pointed out.

BIBLIOGRAPHY

1. CAMERON, T. W. M. 1925 The cestode genus *Mesocestoides* Vaillant. J. Helm. 3: 33-44.
2. COATNEY, G. R. 1936 Some notes on cestodes from Nebraska. J. Parasitol. 22: 409.
3. JOYEUX, C. AND BAER, J. G. 1932 Recherches sur les cestodes appartenant au genre *Mesocestoides* Vaillant. Bull. Soc. Path. Exot. 25: 993-1010.
4. MACCALLUM, G. A. 1921 Studies in helminthology. Zoopath. 1: 136-284.
5. MUELLER, J. F. 1928 The genus *Mesocestoides* in mammals. Zool. Jahrb., Syst. Abt. 55: 403-418.
———1930 Cestodes of the genus *Mesocestoides* from the opossum. Am. Midland Naturalist 12: 81-90.
6. WITENBERG, G. 1934 Studies on the cestode genus *Mesocestoides*. Arch. Zool. Ital. 20: 467-508.



A STUDY OF TEMPERATURE EFFECTS ON GREGARINES OF *TENEBRIO MOLITOR* LARVAE*

SISTER MARY MURIEL MACDOUGALL

Gregarines in mealworms have been the objects of many investigations—Pfeffer (1910), Mühl (1921), Daniels (1938), and others—but these studies have been confined chiefly to the morphology or development of various species and have not been concerned with the effects of any physico-chemical variable on these parasites. Von Brand (unpublished) used temperature as a means of defaunating *Tenebrio molitor* from their gregarines. Reyer (1937), referring to von Brand's investigations, assumes that high temperature merely hastened the encystment of the parasites and did not kill them. It seemed of interest to study this mechanism of eliminating the parasites more intensively and to extend the investigations also to the spore stages.

MATERIALS AND METHODS

The gregarines used in this study were obtained from the intestines of mealworms and comprised the three species described and illustrated in the papers of Mühl (1921), and Daniels (1938), namely, *Gregarina steini*, *Gregarina cuneata*, and *Gregarina polymorpha*. The predominant form was *Gregarina polymorpha*; and since the reaction to temperature was apparently quite similar in all three species, it was not deemed necessary to keep separate records for each species. The infection rate of the mealworms used ranged in the different batches from 66 to 99 per cent. In examining the hosts for parasites, the entire alimentary canal of the animal was removed, crushed on a slide in 0.65 per cent saline solution, and observed under the microscope. For routine examination these fresh slides proved entirely satisfactory. In some instances, however, the parasites were stained with borax-carmin.

In conducting the defaunation experiments by the incubation method the mealworms were placed in sterile culture dishes and put in an incubator at $37.5 \pm 1^\circ \text{C}$ for varying periods of time. A small piece of dampened paper towelling added to each dish furnished sufficient moisture. At the close of the incubation period the hosts were transferred to a sterile culture dish and kept at room temperature until dissected. In carrying out experiments in which it was necessary to maintain higher

Received for publication, August 18, 1941.

* A contribution from the Department of Biology, The Catholic University of America, Washington, D. C. This paper, prepared under the direction of Dr. Theodor von Brand, is based on the author's dissertation submitted in partial fulfillment of the requirements for the degree of Master of Science.

temperatures for periods of 15 or 20 minutes, the procedure was somewhat changed. It was deemed unsatisfactory to place the hosts in air at higher temperatures, since for the short periods wanted there was no guarantee that the heat would penetrate the worms. Therefore, a simple water bath was constructed and used for these experiments. The mealworms were immersed in water in a 200-ml Erlenmeyer flask, which was closed with a one-hole stopper into which a thermometer had been inserted. This thermometer registered the temperature of the water which surrounded the mealworms. The flask was fastened to an iron stand and set into a water bath heated to the desired temperature. It was found convenient to use this same method in testing the viability of the gregarine spores in infected bran.

EXPERIMENTAL DATA

The first step was to attempt defaunation experiments on the host larvae by means of the incubation method as von Brand had done. The objective was to free the mealworms from their parasites without causing injury to these hosts. Fig. 1 gives a graphic representation of all the results obtained when ten different batches of mealworms were incubated at 37.5° C for periods ranging from one to six days. Of the 541 experimental animals incubated 51 died, 75 pupated, and 415 were examined. In the graph the percentage of parasite-free hosts is plotted against the incubation period as the abscissae. This curve shows that an incubation period of six days is sufficient to completely free the mealworms from their parasites.

Tenebrio larvae were then submitted to higher temperatures for shorter periods of time, and moist heat was used instead of dry heat. This difference affects, of course, only the hosts, since the gregarines in the intestine live always in moist surroundings. The heat applied to them in the incubation experiments must also be regarded as moist heat. The control group used in this test showed an infection of 85 per cent. Fifty experimental animals were kept immersed in water at 40° C for 15 minutes. The temperature was maintained by means of the water bath previously described. When the hosts first came in contact with the warm water they became unusually active, and twisted and squirmed energetically in an attempt to crawl up the side of the flask and the stem of the thermometer. This pronounced activity gradually diminished and after about 10 minutes only a few of the worms showed any motility and those few were very lethargic. At the end of 15 minutes all motility ceased and the larvae were removed from the flask and placed in a dry, sterile, culture dish. The entire batch of worms was inactivated. By the next morning, however, most of the larvae had revived, although many of them were more lethargic in their movements than usual. When

examined, it was found that the infection rate had dropped from 85 to 17 per cent. The marked reaction which the hosts showed to the moist heat in this experiment led to the assumption that they would not be able to withstand a temperature much higher than that which killed their parasites. In order to determine the thermal death-point of the hosts under these conditions, 50 worms were immersed in water at 42.5° to

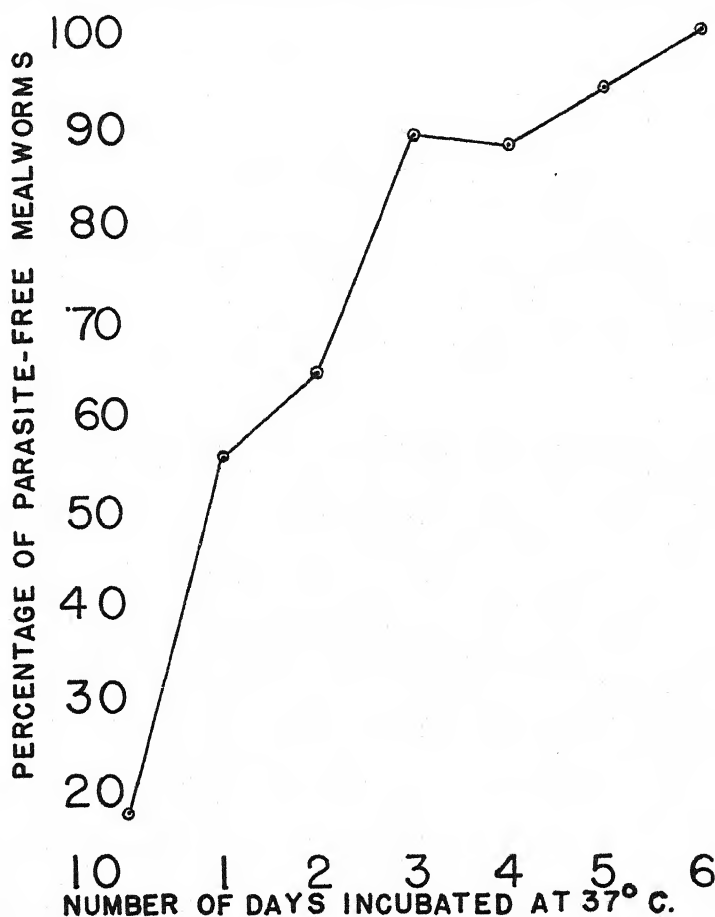


FIG. 1. Defaunation of mealworms kept at 37.5° C.

43.5° C and this temperature maintained for a 15-minute period. Inactivation of the larvae was observed shortly after immersion, and not one of these hosts revived. In other words, the thermal death-point of the gregarine trophozoite, when death is achieved by a short exposure to high temperatures, is only about 3° lower than that of their hosts.

The experiments mentioned so far show that a temperature of 40° C is lethal to the gregarines but they are not sufficient to decide whether or

not Reyer's hypothesis concerning the action of a temperature of 37° C is correct. The decisive test had to be preceded by reinfection experiments.

One hundred and thirty-five mealworms were first defaunated by incubation for one week at 37.5° C, and after removal from the incubator kept at room temperature and fed bran containing the spores of the gregarines for periods of 7, 10, and 14 days. The spore-containing bran was obtained from the jar in which the stock culture of infected *Tenebrio* larvae was kept. The average reinfection which took place in the three feeding periods was 91 per cent. In each lot examined in this series very small gregarines were present which indicated the expected "new infection," but together with them large ones appeared in some of the larvae, which might be interpreted as an "old infection." In order to account for these large gregarines another test was made. This time 29 defaunated larvae were put into spore-infected bran for only three days. Examination of the hosts at the close of the three day feeding period showed 25 parasite-free larvae and only one infected larva. (Three of the lot were dead.) The one positive finding revealed a very sparse infection of only small gregarines. It may be supposed that actually more mealworms were already infected, but that the parasites were still in the intracellular stage and thus escaped detection. Therefore, it seems likely that the large gregarines noted in the earlier reinfection experiments were due to the fact that some of the larvae may eat spores on the first day that they are in the infected bran and so become parasitized earlier than some others in the same lot that may not have come in contact with the spores until several days later.

The fact that mealworms were so easily reinfected allows a further, and, it is believed, a decisive test concerning Reyer's hypothesis. Fifty worms, defaunated in the usual manner, were put into spore-infected bran for one week. They were then removed from the bran and 20 of the lot were examined. Seventeen of these showed a heavy infection of both large and small gregarines, two showed a heavy infection of only small gregarines, one was negative. The remaining 30 of this lot were not examined but were put into a sterile culture dish and incubated at 37.5° C for a period of four days. If a temperature of 37.5° C does not kill the gregarines but merely hastens the life cycle, as Reyer assumed, the incubation period of four days with newly reinfected larvae should not eliminate the parasites, but merely allow the young infections to grow to the size of the old infections. However, when this lot was examined, 29 were found to be negative; one had died. The legitimate conclusion is that the gregarine trophozoites had actually been killed.

These experiments conducted on the trophozoite stage of the gregarines were followed by a series of experiments designed to test the viability

of the spore stage at various temperatures. The criterion of spore viability was to be the number of infections observed in larvae fed upon the spore-containing bran after it had been subjected to varying temperatures. Bran taken from the infected stock culture, and containing a quantity of the granular, sandy excreta in which the gregarine spores occur, was placed in a 200-ml Erlenmeyer flask. The flask was equipped with a thermometer, the bulb of which was placed in the center of the bran. The flask was immersed in a water bath and the desired temperatures maintained for periods of 15 or 20 minutes after the bran had reached the intended temperature. At the end of this time the bran was brought to room temperature and transferred to a sterile culture dish. Mealworms which had been previously defaunated by the incubation method were put into this bran and kept at room temperature for a period of one week, at the end of which time they were examined for the presence of gregarines. The results of this series are summarized in Table 1. Temperatures of 49°–50° C and 60°–64° C did not kill the

TABLE 1.—*Spore viability tested by dry heat*

Temperature to which spores were heated	Number of hosts fed on test bran for 7 days	Remarks	Number of hosts showing gregarine infection	Number of non-infected mealworms	Percentage of hosts infected by viable spores
49–50° C–15 min.	54	2 pupated; 3 dead; 49 exam.	39	10	80%
60–64° C–15 min.	39	10 dead; 29 exam.	22	7	76%
68–70° C–20 min.	50	50 exam.	1	49	1%
95–96° C–20 min.	56	10 dead; 46 exam.	4	42	9%

spores, but they were destroyed if exposed for 20 minutes to 68°–70° C.

Dry spore-infected bran was also submitted to incubation at 37.5° C for periods of 50 and 90 days, but the spores were still viable as 60 per cent of the parasite-free mealworms fed on this bran became infected in the first instance, and 73 per cent in the second.

In order to decide whether moist heat would prove more detrimental to the spores than dry heat, infected bran was placed in a flask—in the same manner as described above—and covered with water heated to the same temperature as that of the water bath in which the flask was placed. This temperature varied in each test and was maintained for periods of 15 or 20 minutes. After heating, the bran was removed from the flask, spread out in a pan and allowed to dry in air. When thoroughly dry, the bran was ground in a mortar in order to break up the large clumps formed when the bran was wet. This bran was then put in a sterile culture dish and mealworms which had been previously defaunated were put in the dish to feed upon the bran for a period of one week. This series is summarized in Table 2. A temperature of 39°–40° C for 20 minutes

TABLE 2.—*Spore viability tested by moist heat*

Temperature to which spores were heated	Number of hosts fed on test bran for 7 days	Remarks	Number of hosts showing gregarine infection	Number of non-infected mealworms	Percentage of hosts infected by viable spores
39–40° C–20 min.	28	1 dead; 27 exam.	20	7	74%
54–55° C–20 min.	40	2 dead; 38 exam.	none	38	—
63–64° C–20 min.	50	5 dead; 45 exam.	none	45	—
48–50° C–15 min.	43	2 pupae; 33 exam. 10 dead.	none	33	—

had no detrimental effect on the spores but temperatures of 48° to 50° C and above killed the spores.

DISCUSSION

Cleveland (1924) was able to completely defaunate termites (without injuring these hosts) by incubating them at 36° C for a period of 24 hours. Von Brand (unpublished) sterilized *Tenebrio* larvae by incubating them at 37° C for a period of five days. In this study, complete defaunation was accomplished by a six-day incubation period at 37.5° C. In contrast to Reyer's opinion, these experiments prove that this temperature actually kills the trophozoite stage of the gregarines and does not merely accomplish defaunation by causing the vegetative stage to encyst. The fact that gregarine trophozoites disappear from the mealworms after the latter have been immersed for a short time into water of 40° C is doubtless due to a lethal influence of this temperature. The mealworms become, as mentioned above, immotile, but recover after a certain time. The suspicion that the gregarines were not killed by this temperature, but by asphyxiation which might result from a curtailment of breathing activities of the hosts during the period of immobility, was disproved by von Brand. He (personal communication) subjected mealworms to anaerobic conditions lasting four hours, during which time they ceased all movements. After recovery they showed only an insignificant drop in infection rates as compared to the control group.

The experiments testing spore viability bring out the fact that the spores could be killed by moist heat at 48°–50° C for 15 minutes, or by dry heat at 68°–70° C for 15 minutes. In comparing these results with those of other investigators who made similar studies on the viability of cysts of intestinal protozoa, we find that there is considerable variation in viability even among parasites living in the same environment. Boeck (1921) made extensive studies on the resistance of human intestinal cysts to moist heat with five minutes exposure at 48°–52° C. When exposed to temperatures higher than 52° C, the number of dead *Endamoeba histolytica* cysts increased with each increase of temperature until all of them were dead at the thermal death-point of 68° C. *Endamoeba*

coli cysts displayed a high degree of resistance from 52° to 64° C, and their thermal death-point was not reached until they had been exposed for 5 minutes at 76° C. The cysts of *Iodamoeba bütschlii* were less resistant, as large numbers died after being exposed to temperatures above 56° C; and the thermal death-point was reported to be 64° C for five minutes. *Endolimax nana* cysts also had a thermal death-point of 64° C, as did *Giardia intestinalis* cysts. *Chilomastix mesnili* cysts proved to be almost as resistant to heat as were those of *Endamoeba coli*, as their thermal death-point was reached after a 5-minute exposure to 72° C.

Reinhardt and Becker (1933) subjected the cysts of *Eimeria miyairii* to moist heat maintained by means of an electrically controlled water bath. They found that the unsporulated oöcysts were killed in 15 seconds at a temperature of 53° C and in 24 hours at a temperature of 41° C.

Ellis (1938) tried the effect of dry heat exposure (incubation method) on sporulated and unsporulated oöcysts of *Eimeria tenella*. Sporulated oöcysts became non-viable after one day exposure to a temperature of 39° C. At this same temperature unsporulated oöcysts did not sporulate. Neither did sporulation occur when the cysts were exposed to temperatures as low as 4.4° C or as high as 37° C for 48 hours; however, the viability of the cysts was retained as sporulation promptly occurred when these cysts were placed at 21.1° C.

SUMMARY

1. An incubation period of six days at 37.5° C is sufficient to completely defaunate mealworms from their parasitic gregarines and this temperature actually kills the gregarine trophozoites and does not merely hasten their encystment.
2. The thermal death-point of the trophozoite stage is 40° C when exposed to moist heat for 15 minutes.
3. The thermal death-point of this parasite is only 2° to 3° lower than the thermal death-point of its host.
4. The defaunated hosts may be reinfected by feeding them bran containing gregarine spores.
5. Spore viability is destroyed when gregarine spores are exposed to dry heat at a temperature of 68°–70° C for 20 minutes; or when they are exposed to moist heat at a temperature of 48°–50° C for 15 minutes.
6. Spore viability is not affected by an incubation period of ninety days dry heat at 37.5° C.

BIBLIOGRAPHY

- BOECK, W. C. 1921 The thermal deathpoint of the human intestinal protozoan cysts. *Am. J. Hyg.* 1: 365–387.
- CLEVELAND, L. R. 1924 The physiological and symbiotic relationships between the intestinal protozoa of termites and their hosts, with special reference to *Reticulitermes flavipes* Kollar. *Biol. Bull.* 46: 177–225.

- DANIELS, M. I. 1938 A cytological study of the gregarine parasites of *Tenebrio molitor*, using the ultracentrifuge. Quart. J. Micr. Sc. 80: 293-320.
- ELLIS, C. C. 1938 Studies on the viability of the oocysts of *Eimeria tenella*, with particular reference to conditions of incubation. Cornell Vet. 28: 267-274.
- MÜHL, D. 1921 Morphologie und Physiologie der Mehlwurmgregarinen. Arch. Protistenk. 43: 362-413.
- PFEFFER, E. 1910 Gregarinen im Darm der Larve von *Tenebrio molitor*. Arch. Protistenk. 19: 107-118.
- REINHARDT, J. F. AND BECKER, E. R. 1933 Time of exposure and temperature as lethal factors in the death of the oocysts of *Eimeria miyairii*, a coccidian of the rat. Iowa State Coll. J. Sc. 7: 505-510.
- REYER, W. 1937 Infektionsversuche mit *Barruxi schneideri* an *Lithobius forficatus*, insbesondere sur Frage der Sexualität der Coccidiensporozoiten. Z. Parasitenk. 9: 478-522.



A NEW SPIRUROID NEMATODE, *THELAZIA BUTEONIS*,
FROM SWAINSON'S HAWK*

KARL E. HERDE

In April 1939 a Swainson's hawk, *Buteo swainsoni* Bonaparte, 1838, collected near Tahlequah, Oklahoma, was found to contain in the orbit of one eye a male and six female specimens of an apparently new thelaziid nematode. The worms were preserved in 10 per cent formalin. Later they were washed in water, partially dehydrated in alcohol, and transferred to glycerin for study.

Thelazia buteonis n. sp.

(Figs. 1-4)

Description: Body slender, transparent, and slightly milky or colorless in life. Cuticle marked by numerous (74-86 per mm of body length) annular constrictions which are very distinct anteriorly but gradually fade out toward the rear and become unrecognizable at a level slightly in front of the anus—optical margins of anterior two-thirds of body slightly serrate in appearance because of these annulations. Mouth and buccal cavity roughly circular in en face view. Cephalic papillae, present in the number and arrangement characteristic of the genus, include, besides the prominent amphids, six minute internals, medium-sized dorsodorsals and ventro-ventrals, and large laterodorsals and lateroventrals. Nerve ring prominent, near middle of esophagus. Deirids somewhat behind nerve ring.

Female: Measurements from 5 mature preserved specimens under cover glass pressure. Length 13.0 (12.1-13.9) mm, width near middle of body 605 (471-648) μ . Buccal cavity—length 35 (28-41) μ , width 50 (41-55) μ . Esophagus—length 562 (506-615) μ , greatest width 84 (80-87) μ . Distance from anterior end—nerve ring 286 (275-300) μ , vulva 575 (522-647) μ , deirids 391 (388-413) μ . Tail broadly conical with a rounded tip; 210 (187-233) μ long. Vulva near level of posterior end of esophagus, being sometimes slightly in front of and sometimes slightly back of this point.

Male: The single preserved specimen was measured under cover glass pressure. Length 11.1 mm, width near middle of body 573 μ . Buccal cavity 27 μ long and 45 μ wide. Esophagus 487 \times 87 μ . Nerve ring 258 μ from front end. Tail broadly rounded at tip, 173 μ long. Spicules—right 204 \times 36 μ , stout, heavily chitinated, and with a rugged surface; left 1658 \times 9 μ , delicate and acicular. Caudal papillae include 8 left, 9 right, and 1 median preanals; 1 right para-anal; and 7 pairs of rather symmetrically arranged postanals.

Host: *Buteo swainsoni* Bonaparte, 1838.

Habitat: Orbit of eye.

Locality: Tahlequah, Cherokee County, Oklahoma.

Specimens: Four females and one male in alcohol, in U. S. Nat. Mus. Helm. Coll., No. 36804 (cotypes).

The genus *Thelazia* Bosc, 1819, contains 31 previously known species all of which appear normally to be parasitic about the eyes of birds or mammals. The new species was studied in comparison with descriptive

Received for publication, September 4, 1941.

* A contribution from Northeastern State College (Tahlequah, Oklahoma) and (No. 95) the Zoological Laboratory, Oklahoma Agricultural and Mechanical College; prepared under the direction of R. Chester Hughes.

data on all of the others. The writer's sources of information were the compilatory accounts of Cram (1927) and Price (1931) for the older forms and the respective original descriptions of Baylis (1920 and 1934b), Dubinina (1937), Ershov (1928), Hsü (1933 and 1935), Oser-skaja (1931), Price (1930), Railliet and Henry (1910a and 1910b), Sandground (1933), Schuurmans Stekhoven (1937), Smit and Noto-Soediro (1930), and Wehr (1930) for the several forms whose specific names first appear in them.

The 20 previously described avian species of *Thelazia* differ from *T. buteonis* n. sp. as severally indicated below:

T. annamensis Sandground, 1933, *T. campanulata* (Molin, 1858) Railliet and Henry, 1910, *T. cholodkowskii* Skrjabin, 1922, *T. cirrura* (Leidy, 1886) Railliet, 1916, *T. dactylon* (Breinl, 1913) Travassos, 1918, *T. digitata* Travassos, 1918, *T. digiticaudata* Schuurmans Stekhoven, 1937, *T. longicauda* Sandground, 1933, *T. lutzii* Travassos, 1918, *T. nyctardeae* Dubinina, 1937, *T. stereura* (Rudolphi, 1819) Railliet and Henry, 1910, and *T. tonkinensis* Hsü, 1935, all have the vulva in the female located cephalad of the anterior end of the intestine by at least one-fourth of the length of the esophagus;

T. anolabiata (Molin, 1859) Railliet and Henry, 1910, has finger-like processes that extend into the buccal cavity from the rim of the mouth;

T. aquilina Baylis, 1934b, is considerably larger and has only 4 pairs of postanal papillae in the male; in

T. chui Hsü, 1935, described from a single male specimen, the body is considerably larger, the right spicule is much shorter in proportion both to the length of the body and the length of the left spicule, and a "gubernaculum-like" structure occurs near the cloaca; in

T. chungkingensis Hsü, 1933, the body is smaller in both sexes, the tail of the female is relatively shorter, and the male has fewer caudal papillae; in

T. dentifera Sandground, 1933, the tail is much shorter in both sexes, the male has fewer caudal papillae, and the left spicule is less than twice as long as the right;

T. depressa Baylis, 1920, is considerably larger and has the tail in both sexes terminating in a peculiar digitiform process; in

T. papillosa (Molin, 1860) Railliet and Henry, 1910, the body is more slenderly elongate, the neck is provided with delicate cuticular spines, the tail of the female is more bluntly rounded, and the male has fewer caudal papillae; and in

T. philippinensis Wehr, 1930, the male has fewer postanal papillae, the length of the left spicule is greater in proportion to that of the right, and the tail of the female is relatively much shorter.

The 11 mammalian species differ from the new form as follows: in *T. alfortensis* Railliet and Henry, 1910a, *T. californiensis* Price, 1930, *T. erschowi* Oserskaja, 1931, *T. floresiana* Smit and Noto-Soediro, 1930, *T. gulosa* Railliet and Henry, 1910a, *T. lachrymalis* (Gurli, 1831) Railliet and Henry, 1910, *T. leesei* Railliet and Henry, 1910a, and *T. rhodesii* (Desmarest, 1828) de Blainville, 1828, the vulva of the female is situated caudad of the anterior end of the intestine by a distance equal to at least one-third of the length of the esophagus;

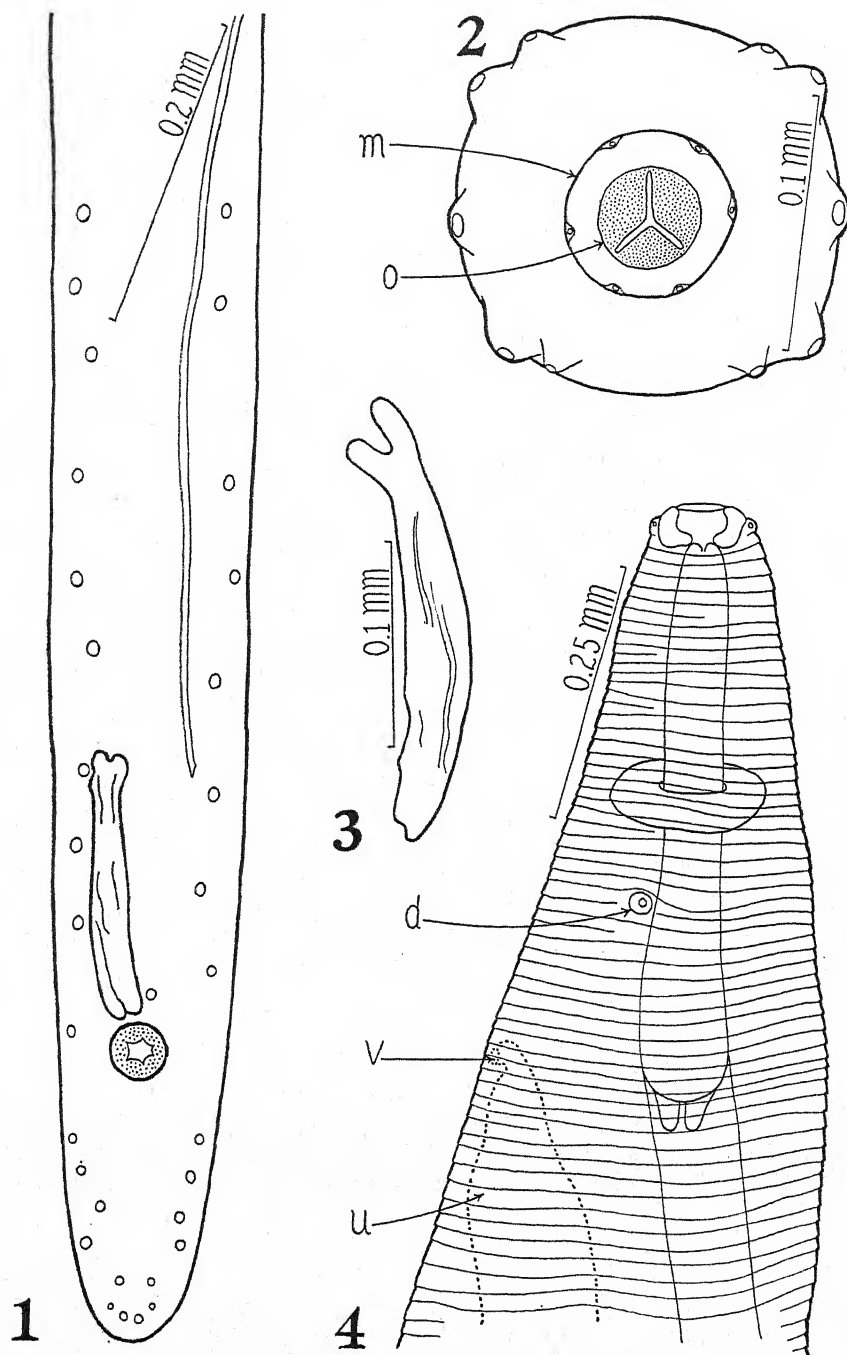
T. callipaeda Railliet and Henry, 1910b, has only 2 pairs of postanal papillae in the male; and in

T. iheringi Travassos, 1918, and *T. skryabini* Ershov, 1928, the males have more numerous preanal and fewer postanal caudal papillae.

Thelazia depressa was originally found in a mongoose but Baylis (1934a) regards it as being normally a parasite of vultures.

BIBLIOGRAPHY

- BAYLIS, H. A. 1920 Notes on some parasitic worms from East Africa. *Ann. and Mag. Nat. Hist.* 6: 283-295.
 ——— 1934a Miscellaneous notes on parasitic worms. *Ibid.* 13: 223-228.
 ——— 1934b Some spirurid nematodes from Queensland. *Ibid.* 14: 142-153.
 CRAM, E. B. 1927 Bird parasites of the nematode suborders Strongylata, Ascariata, and Spirurata. *Bull. U. S. Nat. Mus.* 140: 1-465.
 DUBININA, M. 1937 Parazitofauna kvakvy *Nycticorax nycticorax* L. i ee izmeneniia v sviazi s migratsiei khoziaina. *Zool. Zhurnal* 16: 547-573.
 ERSHOV, V. S. 1928 Teliazioz glaz krupnogo rogatogo skota v SSSR (Die Thelasiose des Rindes in der Union SSR). *Trudy Gosudarstv. Inst. Eksper. Vet.* 5: 12-39.
 HSÜ, H. F. 1933 On some parasitic nematodes collected in China. *Parasitology* 24: 512-541.
 ——— 1935 A study of some Strongyloidea and Spiruroidea from French Indo-China and of *Thelazia chungkingensis* Hsü, 1933 from China. *Z. Parasitenk.* 7: 579-600.
 OSERSKAJA, B. N. 1931 *Thelazia erschowi* n. sp., ein neuer Nematode des Schweines. *Tierärztl. Rundschau* 37: 656-657.
 PRICE, E. W. 1930 A new nematode parasitic in the eyes of dogs in the United States. *J. Parasitol.* 17: 112-113.
 ——— 1931 A note on the occurrence of eyeworms in dogs in the United States. *North Am. Vet.* 12(11), 6 pp.
 RAILLIET, A. AND HENRY, A. 1910a Les thélazies, nématodes parasites de l'oeil. *Compt. Rend. Soc. Biol.* 68: 213-216.
 ——— 1910b Nouvelles observations sur les thélazies, nématodes parasites de l'oeil. *Ibid.* 68: 783-785.
 SANDGROUND, J. H. 1933 Report on the nematode parasites collected by the Kelley-Roosevelts expedition to Indo-China with descriptions of several new species. *Z. Parasitenk.* 5: 542-583.
 SCHUURMANS STEKHOVEN, J. H. 1937 Parasitic Nematoda. In "Exploration du Parc National Albert. Mission G. F. de Witte (1933-1935)," Bruxelles 4: 1-40.
 SMIT, H. J. AND NOTO-SOEDIRO, R. 1930 Een worm uit den conjunctivaalzak van het paard. *Nederl.-Ind. Blad. Diergeneesk.* 42: 601-606.
 WEHR, E. E. 1930 New species of bird nematodes from the Philippine Islands. *J. Parasitol.* 17: 80-84.



Thelazia buteonis n. sp. All figures made with the aid of a camera lucida.
 d—deirid; m—rim of mouth; o—front end of esophagus; u—uterus; v—vulva.
 FIG. 1. Tail of male. Ventral view. FIG. 2. Anterior end. En face view.
 FIG. 3. Right spicule. FIG. 4. Anterior end of female. Sinistral view.

RESEARCH NOTES

OCCURRENCE IN THE HORSE OF TWO PARASITES OF CATTLE, *OSTERTAGIA OSTERTAGI* (STILES, 1892) AND *COOPERIA* *ONCOPHORA* (RAILLIET, 1898)

Unusual parasites, normally inhabiting the alimentary tract of ruminants, in particular cattle, were met with three times in the horse when a large number of horse stomachs and duodenums were examined for parasites.

Case 1 was a four-year-old horse. The stomach showed signs of a chronic hypertrophic gastritis with several circumscribed adenomatous changes in the mucosa, which were due to the presence of numerous *Trichostrongylus axei* (Cobbold, 1879), a parasite already known to occur in both horses and ruminants. In addition, two unfertilized females of the genus *Ostertagia* were found in the stomach, both apparently belonging to the species *O. ostertagi* (Stiles, 1892), the so-called medium stomach worm of the ox. (One of the females was somewhat injured, so that in this case the diagnosis of the species had to be made with some reservation.)

Case 2 was a fifteen-year-old horse that had died of chronic gastroenteritis. The stomach and the duodenum were markedly inflamed. A slight infection with *Trichostrongylus axei* was present in the stomach. Furthermore, 14 mature specimens (5 males and 9 females) of *Ostertagia ostertagi* (Stiles, 1892) and one mature male of *Cooperia oncophora* (Railliet, 1898), a parasite of the small intestine of the ox, were found in the duodenum, while a female of *O. ostertagi* occurred in the stomach.

Case 3 was a one-and-a-half-year-old filly coming from the same pasture as case 1. Its stomach also showed the characteristic changes due to an abundance of *Trichostrongylus axei*. In addition, 5 mature *Ostertagia ostertagi* were found, viz., 3 males and 2 females, 2 of which were in the stomach, the remainder in the duodenum.

So far as the authors are aware this is the first record from the horse of two parasites, otherwise so typical of ruminants, particularly cattle, as are *O. ostertagi* and *C. oncophora*. It may be added that the size of the specimens found in the horses was in all cases nearly the same as in their natural hosts. The male of *C. oncophora* measured about 6.5 mm, the males of *O. ostertagi* from 5.0 to 6.5 mm and the females of *O. ostertagi* from 5.5 to 8.0 mm. Almost all females of *O. ostertagi* in cases 2 and 3, where males also were present, contained typical eggs in the morula stage.—HANS ROTH AND N. O. CHRISTENSEN, *Department of Special Pathology, Royal Veterinary and Agricultural College, Copenhagen* (Director: Professor H. C. Bendixen).

ORAL TRANSMISSION OF *PLASMODIUM CATHEMERIUM* BY MEANS OF TISSUES

Four years ago, an experiment was performed by the author in order to determine whether the exoerythrocytic stages of *Plasmodium cathemerium* in canaries could be transmitted by the oral route. The result was positive in one of four cases. Since an abrasion of the alimentary canal in the process of inoculation was a possible explanation of the positive result, this experiment was not reported at that time. The present report seems justified, however, because of the recent interest in the transmission of *Plasmodium* by the oral route (Shortt & Menon, 1940, J. Malar. Inst. Ind.; Beltran & Larenas, 1941, Rev. Inst. Salub. Enferm. Trop.; and Young, 1941, Pub. Health Rep. U. S. Pub. Health Serv.). The host, the species of *Plasmodium*, the use of tissue and the presence of exoerythrocytic stages in the inoculum makes the present experiment different from those previously reported.

Canary 627 died Nov. 18, 1937, from an infection with the wood thrush strain

of *Plasmodium cathemerium*. Its spleen, which showed both erythrocytic and exoerythrocytic stages, was macerated in citrated salt solution to make up a volume of 360 mgm. This mixture was administered into the crop of canary 637 by the use of a small catheter attached to a syringe. After an incubation period of 9 days, *Plasmodium* was observed in the peripheral blood of canary 637. The patent period of this infection lasted for five days and terminated in death. At autopsy, both erythrocytic and exoerythrocytic stages were observed in the lung, spleen, liver, bone marrow and brain of canary 637.

This experiment was repeated using three more canaries inoculated orally and a fourth, the control, inoculated intramuscularly. A portion of liver, the whole brain and the whole spleen of canary 636 (which died Nov. 28, 1937) were suspended separately in citrated salt solution. Each suspension made up a volume of about 400-460 mgm, and was administered orally into canaries 652, 653 and 654, respectively, using the same method as had been employed for canary 637. The control canary, 655, was inoculated intramuscularly with heart's blood from canary 636. This blood contained about 3,000 parasites per 10,000 erythrocytes; the tissues showed numerous erythrocytic and exoerythrocytic stages. The erythrocytes contained only uninucleate stages of *Plasmodium*, whereas the exoerythrocytic forms were represented by various stages from uninucleated stages to "ripe" schizonts apparently ready for, or in the process of, segmentation.

The control canary, 655, died from the infection 8 days after its inoculation. The other three birds survived and showed no parasites for a period of three months. After this period, one (canary 652) was killed. Its tissues were examined and found negative for malarial infection. The other two were reinoculated intravenously with the same strain of *P. cathemerium* and both died from the infection 13 days later, confirming the previous observation that they had not been infected by the oral route.

To summarize, tissues of canaries containing erythrocytic and exoerythrocytic stages of the wood thrush strain of *Plasmodium cathemerium* were administered by mouth to four canaries on two different occasions. One canary developed an infection. Parasites were observed in its peripheral blood 9 days after oral administration and exoerythrocytic stages were found in its tissues at autopsy 14 days after inoculation. The other three canaries remained uninfected: their inocula contained various exoerythrocytic stages and only mononuclear erythrocytic stages of development.—FRUMA WOLFSON, *Department of Protozoology, School of Hygiene and Public Health, Johns Hopkins University.*

THE INTRAVENOUS INOCULATION OF SPOROZOITES OF *PLASMODIUM GALLINACEUM*

In 1936 Wolfson (J. Parasitol. 22: 291-292) reported the first case of bird malaria, *Plasmodium praecox*, transmitted by intravenous injection of sporozoites in the canary. Three birds were inoculated with 150 mgm of saline-citrate solution, to which a small amount of sterile bovine serum was added. Each bird received sporozoites from about two infected *Culex pipiens*. In one of the canaries the result was negative; in the other two infections of different intensities appeared on the 10th day. Wolfson mentioned the unsuccessful experiments of previous workers.

In 1937, Warren and Coggeshall (Am. J. Hyg. 26: 1-10) reported the intravenous inoculation of canaries with sporozoites of *P. cathemerium*, in saline solution, from infected salivary glands of *C. pipiens*; they said they had used this method for several months with "continuous success."

In this paper we report the intravenous injection of sporozoites of *P. gallinaceum* into two Leghorn chickens, each more than four months old.

Infected salivary glands of *Aedes aegypti* were crushed in a drop of Locke's solution, and suspended in the same fluid. The injection was performed in a wing vein. In order to insure that no sporozoites were accidentally injected outside the blood vessel, the syringe was charged without needle, a small air bubble was allowed

to enter the tip of the syringe, which was then carefully cleaned. A clean needle was inserted and no pressure exerted until it was ascertained that the needle was in the vein; the passage of the air bubble inside the blood vessel proved that the needle was correctly placed.

In the first chicken (Ga 168) the animal received sporozoites from the salivary glands of one mosquito. On the 6th day after the inoculation the blood was still negative, but on the 10th day parasites were found in the circulating blood in the proportion of 1,400 parasites per 10,000 erythrocytes.

In the second chicken (Ga 184) the animal received sporozoites from the salivary glands of about 1.5 mosquitoes. Parasites were found in the circulating blood on the 11th day after the injection, when their number was 9 per 10,000 erythrocytes.

The prepatent period after the injection of sporozoites intravenously seems to be the same as that observed after natural or artificial inoculation by subcutaneous or intramuscular routes. Our records (eight cases) vary from seven to eleven days. This similarity in the period of incubation after inoculation with sporozoites by different routes, was observed by Warren and Coggeshall in their studies with *P. cathemerium*.

The possibility of causing infections by intravenous injections of sporozoites is interesting, because of the gap existing in our knowledge of the primary stages of malarial infections naturally caused by mosquitoes. It is desirable to establish if all the species of plasmodia have or have not the possibility of being transmitted by intravenous injection of sporozoites.

The studies of James and associates (1927, Tr. Royal Soc. Trop. Med. and Hyg. 21: 233-236) show that human malaria can be induced by intravenous inoculation of sporozoites. The papers of Wolfson, and Warren and Coggeshall, and our experiments here reported, show that the transmission of avian malaria by intravenous injection of sporozoites is possible in the canary with *Plasmodium praecox* and *P. cathemerium*, and in the chicken with *P. gallinaceum*.—ENRIQUE BELTRAN and LUIS VARGAS, *Instituto de Salubridad y Enfermedades Tropicales, México, D. F.*

AMERICAN SOCIETY OF PARASITOLOGISTS

SEVENTEENTH ANNUAL MEETING, DALLAS, TEXAS

DECEMBER 29 TO 31, 1941

Minutes of the Seventeenth Annual Business Meeting

The seventeenth annual business meeting of the Society was held at Hotel Baker on December 30, 1941, following the annual Parasitologists' Luncheon, which was attended by 95 members and guests. The meeting was called to order at 1:20 PM by President James E. Ackert who introduced the officers of the Society and the five former Presidents who were present, Drs. Henry B. Ward, W. W. Cort, W. A. Riley, H. W. Stunkard and D. H. Wenrich.

I. REPORTS OF OFFICERS

1. The report of the Secretary was presented. As of December 10, 1941, there were 447 members in good standing and 65 who were delinquent in dues for from one to three years, making a total membership roll of 512. Thirty-eight new members were elected during 1941. The deaths of the following members occurred during the past year:

John E. Guberlet, Professor of Zoology at the University of Washington, December 30, 1940, at Seattle, Washington.

Charles Wardell Stiles, former President of the Society and Honorary Life Member, January 24, 1941, at Baltimore, Maryland.

Donald Cameron, of Ottawa, Canada, was killed in service with the Royal Canadian Air Force, June, 1941, at Malta.

The Secretary also reported the following actions of the Council at its meeting held on December 28, 1941:

(1) Acceptance of the recommendation of the Secretary that, in the future, simultaneous sessions be scheduled, if necessary, to fit the program into a three-day meeting period.

(2) Adoption of the report of the special nominating committee (Dr. W. W. Cort, Chairman, Drs. F. C. Bishopp, A. C. Chandler, E. C. Faust, J. F. Kessel, Benjamin Schwartz, H. W. Stunkard, D. H. Wenrich and J. E. Ackert, ex officio) which recommended that the present Editorial Committee of the JOURNAL OF PARASITOLOGY, Dr. N. R. Stoll, Chairman, Drs. W. H. Taliaferro and W. A. Riley, be elected for an additional term of one year beginning January 1, 1943.

(3) Passage of a unanimous vote of thanks to Dr. N. R. Stoll and his collaborators, Dr. and Mrs. M. S. Ferguson and Dr. George L. Graham, Dr. P. D. Harwood and Dr. A. C. Jerstad, for their painstaking work in preparing and publishing the Index for the first twenty-five volumes of the JOURNAL OF PARASITOLOGY.

(4) Election of Dr. Juan Bacigalupo, Buenos Aires, Argentina, as a Foreign Honorary Member of the Society.

Upon motion the report was accepted and placed on file.

2. The report of the Treasurer was presented by Dr. L. E. Rozeboom. The year's business was summarized as follows:

Credits	
Received for 1941	\$3963.85
Anticipated for 1941	93.60
	<hr/>
	\$4057.45
Debits	
Cost of Journal	\$3300.54
Chm. Ed. Comm. \$ 90.51	
Secretary	187.39
Treasurer	390.39
Miscellaneous ...	10.00
	<hr/>
	678.42
	<hr/>
	3978.96
Balance from regular business	\$78.49

In the estimate of receipts and expenditures for next year it was pointed out (1) that 55 non-member subscribers in enemy and occupied territory would be lost from the subscription list for the JOURNAL; (2) that the costs per page of publishing the JOURNAL would increase approximately 10 per cent; (3) that Council, in order to avoid a deficit, had voted to reduce the estimated number of pages to be published in the JOURNAL during 1942 from 600 to 540 pages. Upon motion, the report, certified as correct by the Auditing Committee, was accepted and placed on file.

II. REPORTS OF COMMITTEES

1. The report of the status of the Secretarial Fund prepared by Dr. N. R. Stoll, Custodian, was read by Dr. L. E. Rozeboom. Upon motion, the report, certified as correct by the Auditing Committee, was accepted and placed on file.

2. The report of the Editorial Committee prepared by Dr. N. R. Stoll, Chairman, was presented by Dr. H. W. Stunkard. Attention was called to the publication of the Index for Volumes 1-25 of the JOURNAL in August, 1941, and all mem-

bers were urged to take the responsibility of seeing to it that their college and departmental libraries secure copies as soon as possible. The sale in 1942 of at least 180 copies at \$2.00 apiece is needed. The report noted that for regular articles the interval between "received for publication" and "date published" increased from an average of 6.5 months in 1940 to 10.7 months in 1941. Seventy-one articles and research notes are on hand (accepted or under consideration) and present acceptances are for the October number. It was also noted that beginning with the February, 1942, issue, reprints of articles will cost an estimated 10 per cent more than the present rates. Upon motion, the report was accepted with a vote of thanks to the Editorial Committee.

3. The report of the Committee for Cooperation with Biological Abstracts was given by Dr. George L. Graham, Chairman. A survey in April, 1941, showed that only 48 members of the Society were subscribers to one or more sections of Biological Abstracts. It was pointed out that the former practice of giving complete taxonomic citations for parasitological articles has been restored, and that abstracts are now appearing more promptly than ever before. Dr. George W. Hunter, III, suggested that abstracts covering medical entomology might be included in Section C without added cost to subscribers. Upon motion, a resolution was voted that the Society go on record as favoring the inclusion of abstracts of articles on medical entomology in Section C of Biological Abstracts.

4. A report of the Committee on the Terminology of Bird Malaria (copy appended) was explained briefly by Dr. R. D. Manwell. Upon motion, the report was accepted for publication in the JOURNAL.

III. REPORTS OF REPRESENTATIVES OF THE SOCIETY

1. As representative on the Council of the American Association for the Advancement of Science, Dr. W. A. Riley gave a brief report of the Council meeting on the preceding day. New York City will be the meeting place of the Association in December, 1942.

IV. NEW BUSINESS

The following amendment of the Constitution proposed at the sixteenth annual meeting in Philadelphia was adopted by unanimous vote: "The Council shall consist of the President, the Vice-president, the Secretary, the Treasurer, *the Chairman of the Editorial Committee*, and eight members elected by ballot from the Society at large." (The phrase italicized represents the change adopted.)

Following the recommendation of the Council it was voted that Dr. George R. LaRue be authorized to organize in the name of the Society a program of parasitology at the summer meeting of the American Association for the Advancement of Science at Ann Arbor, Michigan, June 22-26, 1942, provided that no expense to the Society be incurred.

Dr. T. C. Nelson suggested that as a means of raising money for the Society it might be possible for members to donate extra research slides and material for sale to those wanting to purchase parasitological specimens. The proposal was discussed briefly but no formal action was taken.

The Secretary presented the Council nominations for officers of the Society for 1942, as follows: *President*, Henry E. Meleney; *Vice-president*, Rudolph W. Glaser; *Secretary*, for two years, Oliver R. McCoy; *Members of the Council*, for four years (to succeed E. C. Faust and H. J. Van Cleave), Raymond M. Cable and Willard H. Wright, for three years (to fill the unexpired term of N. R. Stoll), Gilbert F. Otto; *Members of the Editorial Board*, for four years (to succeed W. W. Cort, Benjamin Schwartz and D. H. Wenrich), W. W. Cort, Benjamin Schwartz and Harold Kirby, Jr.; *Representatives on the Council of the American Association for the Advancement of Science*, Asa C. Chandler and Harley J. Van Cleave; *Representatives on the Council of the Union of American Biological Societies*, George L. Graham and Arthur C. Walton. There were no further nominations and upon motion, duly

seconded, the Secretary was instructed to cast one ballot for the nominations as presented.

Upon motion, a vote of thanks was extended to Dr. E. W. Laake, local representative of the Society in Dallas, for his services in making arrangements for the luncheon and the demonstration program.

There being no further business, it was voted at 2:10 PM to adjourn until the next annual meeting in New York City in December, 1942.

Respectfully submitted,

O. R. McCoy, *Secretary*

CHANGES IN MEMBERSHIP OF THE AMERICAN SOCIETY OF PARASITOLOGISTS SINCE JUNE, 1941

New Members Elected

Louis J. Adolphsen	Harold Elishewitz	George Piternick
Robert M. Allen	William G. French	Irving Rappaport
G. F. Auguston	Jacob Frenkel	Nathan W. Riser
John L. Avery	Piero Gallo	Maurice M. Rothman
Gregory Bard	Frans G. Goble	Albert J. Samorodin
Aileen E. Bonestell	Theodore S. Hauschka	Albert J. Singer
William F. Cantrell	Philip A. Hawkins	Enrique G. Vogelsang
José C. M. Carvalho	Leon Jacobs	Joseph J. Volk
Frank H. Connell	John C. Johnson	Robert E. Ware
L. Reid Davis	Edward K. Markell	Albert J. Weathersbee
Willis H. Doetschman	Harry Most	J. Dan Webster
Wilbur G. Downs	Melvin Oosting	
Hilton H. Earle	Felise Pifano	

Resignations

Winterton C. Curtis	Rustum Maluf	Richard P. Strong
Anne Hager	George C. Shattuck	Zeferino Vaz

Deaths

Donald Cameron	Matthew E. Carroll, Jr.	Robert Hegner
----------------	-------------------------	---------------

SUPPLEMENT TO THE REPORT OF THE SEVENTEENTH ANNUAL MEETING OF THE AMERICAN SOCIETY OF PARASITOLOGISTS

REPORT OF THE COMMITTEE ON TERMINOLOGY OF STRAINS OF AVIAN MALARIA

PROPOSALS FOR A UNIFORM TERMINOLOGY OF STRAINS OF AVIAN MALARIA

A recognition of the growing need for some degree of standardization of the terminology of strains of avian malaria led to the appointment by President Wenrich in November, 1940, of a committee to consider this problem.

The need for some attempt at uniformity in terminology has become greater with: (1) the isolation of many strains of avian *Plasmodium* from wild birds, (2) the prevalent practice of the free exchange of such strains among laboratories and investigators, and (3) the discovery that the strains may change in one or more characteristics during laboratory study. Already there is some discrepancy in

designations given in publications to some of the strains by different investigators. It is the belief of the committee that prompt action on the part of malariologists in adopting a satisfactory terminology will avoid much needless confusion in the future.

The problems arising from the use of many strains of avian malaria and from their ability to change in character are greatly different from problems of zoological nomenclature. The former are more like those faced by bacteriologists in designating type cultures and their variants. For this reason the committee has decided that they should avoid as much as possible questions involving zoological nomenclature.

After studying a number of plans the committee has chosen the one which seemed to have the most to offer in convenience and accuracy. It is as follows:

1. Designations will consist of combinations of numerals and letters, beginning with a numeral.

2. The first portion of the designation, consisting of one or more numerals, corresponds to the species of *Plasmodium*. The species for which there are now strains in use will be designated approximately chronologically, i.e., *P. relictum* will be 1, *P. vaughani* will be 2, etc. The committee has no authority to change the specific identification given a strain by the investigator. Likewise, strain designations will have no weight in matters of priority of specific names. The committee will follow the desires of the investigator in manners of zoological classification. One numeral (50) will be reserved for strains not yet identified or described. The designations will be changed when the necessary information becomes available.

3. The letter, or letters, following the first numeral will indicate the original strain. Ordinarily this will apply to strains originating from natural infections of birds or the transmitting hosts. The letters which are already widely used will be preserved wherever possible, e.g., H, which has been used for Hartman's strain of *P. cathemerium*, will be retained. The letters O and I will not be employed because of the chance of confusion with numerals. When more than one letter is used in a designation there is no implied relationship to strains bearing one of these letters singly, e.g., HA is not necessarily related to H.

4. The numerals following the letters of the original strain designation (i.e., the second set of numerals) indicate sub-strains derived from originally isolated strains; thus the gametocyteless strain of *P. cathemerium* discovered by Huff and Gambrell becomes 3D1. It is recommended that sub-strains be designated only (1) when they are actually observed to differ from their parent strains, or (2) when some treat-

TABLE 1.—*Species designations*

Species numeral	Species name	Author	Date
1	<i>relictum</i>	Grassi and Filetti	1891
2	<i>vaughani</i>	Novy and MacNeal	1908
3	<i>cathemerium</i>	Hartman	1927
4	<i>rouxi</i>	Sargent, Sargent and Catanei	1928
5	<i>elongatum</i>	Huff	1930
6	<i>circumflexum</i>	Kikuth	1931
7	<i>polare</i>	Manwell	1934
8	<i>gallinaceum</i>	Brumpt	1935
9	<i>nucleophilum</i>	Manwell	1935
10	<i>hexamerium</i>	Huff	1935
11	<i>oti</i>	Wolfson	1936
12	<i>lophurae</i>	Coggeshall	1938
13	<i>durvae</i>	Herman	1941
50	unidentified or undescribed species		

ment is given the strain which is likely to result in change. Examples of the second type are the isolation of a clone, radical treatment by physical or chemical means, or passage through different hosts.

5. In order not to make the designation too cumbersome, further subdivisions of strains when they need to be made will be indicated by numerals separated from the rest of the designation by a hyphen; thus if a strain is derived from 3H2-1 or 3H2, which differs from its parent strain, it will be given a new terminal number,

TABLE 2.—Designations of original strains

Designation	Host source	Locality	Isolated by	Date of isolation	Other names used	Reference
<i>P. relictum</i> 1 G 1 H 1 M 1 P 1 R 1 T 1 V 1 W	<i>Carpodacus mexicanus frontalis</i> (Mexican finch) <i>Passer domesticus</i> (English sparrow) <i>Columba livia</i> (Pigeon) <i>Planesticus migratorius</i> (American robin) <i>Hylodichia ustulata</i> (Wood thrush) <i>Passer domesticus</i> (English sparrow) <i>Passer domesticus</i> (English sparrow)	Hamburg, Germany Mexico City Syracuse, N. Y. Peru, Nebr. Kansas, Ill. Baltimore, Md. Hampton, Va. New York City	Hewitt Manwell Coatney Huff Wolfson Huff Whitmore	* 1939 1938 1938 1935 1936 1926 1913	German Mexican Var. <i>maifutunum</i> Pigeon Var. <i>maifutunum</i> Matthal Virginian Whitmore	J. Parasitol. 25 Suppl.: 16 (1939) Am. J. Trop. Med. 20: 859 (1940) Am. J. Hyg. 27: 380 (1938) J. Parasitol. 23: 400 (1937) Am. J. Hyg. 25: 177 (1937) Am. J. Hyg. 7: 706 (1927) Bull. Johns Hopkins Hosp. 24: 62 (1918)
<i>P. vaughani</i> 2 R 2 S	<i>Planesticus migratorius</i> (American robin) <i>Sturnus v. vulgaris</i> (Starling)	Syracuse, N. Y. Baltimore, Md.	Manwell Hegner & Wolfson	1934 1937		Am. J. Hyg. 21: 180 (1935) Am. J. Hyg. 28: 437 (1938)
<i>P. cathemertum</i> 3 A 3 B 3 D 3 H 3 M 3 Q 3 S 3 T	<i>Quiscalus quiscula aeneus</i> (Bronzed grackle) <i>Agelaius phoeniceus</i> (Redwing) Sparrow <i>Passer domesticus</i> (English sparrow) <i>Pica pica hudsonia</i> (Magpie) <i>Coccyus erythrophthalmus</i> (Cuckoo) <i>Zonotrichia l. leucophrys</i> (White crowned sparrow) <i>Hylodichia ustulata</i> (Wood thrush)	Paris, Ill. Chino, Calif. Rome, Italy Baltimore, Md. Nebraska South American bird in Chicago Zoo Chino, Calif. Baltimore, Md.	Huff Herman Gingrich Hartman Coatney & Rondabush Huff Herman Wolfson	1933 1933 1934 1924 1937 1941 ? 1936	Grackle Hackett Hartman Magpie Woodthrush or Wolfson	J. Infect. Dis. 57: 315 (1935) J. Infect. Dis. 57: 315 (1935)
<i>P. roulei</i> 4 A	Algerian sparrow	Algeria	Sergeant, Sergeant and Catanel	1928	"X"	Am. J. Hyg. 31C: 26 (1940) Compt. Rend. Acad. Sc. 186: 809 (1928)
<i>P. elongatum</i> 5 A 5 B	<i>Passer domesticus</i> (English sparrow) <i>Quiscalus quiscula aeneus</i> (Bronzed grackle)	Kansas, Ill. Kansas, Ill.	Huff Huff	1932 1933	Taber sparrow Grackle	J. Infect. Dis. 57: 315 (1935) J. Infect. Dis. 57: 315 (1935)

* Obtained by Gingrich in 1931 from Inst. f. Schiffs. u. Tropenhyg.

TABLE 2.—(Continued)

Designation	Host source	Locality	Isolated by	Date of isolation	Other names used	Reference
<i>P. circumflexum</i> 6 A	<i>Melospiza m. melodia</i> (Song sparrow)	Syracuse, N. Y.	Manwell and Goldstein	1935		Am. J. Trop. Med. 19 : 279 (1939)
6 B	<i>Planesticus migratorius</i> (American robin)	Syracuse, N. Y.	Manwell and Goldstein	1935		Am. J. Hyg. 30 : 115 (1939)
6 C	<i>Zonotrichia albicollis</i> (White-throated sparrow)	Syracuse, N. Y.	Manwell and Goldstein	1935		Am. J. Trop. Med. 19 : 279 (1939)
6 D	<i>Zonotrichia albicollis</i> (White-throated sparrow)	Syracuse, N. Y.	Manwell and Goldstein	1935		Am. J. Hyg. 30 : 115 (1939)
6 E	<i>Agelaius phoeniceus</i> (Redwing)	Cape Cod, Mass.	Herman	1936		Am. J. Trop. Med. 19 : 279 (1939)
6 G	<i>Turdus pilaris</i> ("Thrush")	Germany	Kikuth	1931		Centr. Bakt. 121 : 401 (1931)
<i>P. polare</i> 7 A	<i>Petrochelidon l. lunifrons</i> (Cliff swallow)	Westport, N. Y.	Manwell	1934		J. Parasitol. 22 : 412 (1936)
<i>P. gallinaceum</i> 8 A	<i>Gallus domesticus</i> (Domestic chicken)	Ceylon	Brunpt	1935		Compt. Rend. Acad. Sc. 260 : 783 (1935)
<i>P. nucleophilum</i> 9 A	<i>Dumetella carolinensis</i> (Catbird)	Syracuse, N. Y.	Manwell	1934		Am. J. Trop. Med. 15 : 265 (1935)
<i>P. hexameritum</i> 10 A	<i>Melospiza m. melodia</i> (Song sparrow)	Syracuse, N. Y.	Manwell	1935		Am. J. Hyg. 27 : 196 (1938)
10 B	<i>Passerculus sandwichensis</i> <i>savanna</i> (Savannah sparrow)	Syracuse, N. Y.	Manwell	1941		
10 C	<i>Geothlypis t. trichas</i> (Maryland yellowthroat)	Syracuse, N. Y.	Manwell	1941		
10 J	<i>Sialia s. sialis</i> (Bluebird)	Kansas, Ill.	Huff	1934		Am. J. Hyg. 22 : 274 (1935)
<i>P. oti</i> 11 A	<i>Asio otus naevius</i> (E. screech owl)	Glen Rock, Penn.	Wolfson	1936		Am. J. Hyg. 24 : 94 (1936)
<i>P. lophurae</i> 12 A	<i>Lophura l. igniti</i> (Fire-backed pheasant)	Borneo (N. Y. zoo)	Coggeshall	1938	Strain 1	Am. J. Hyg. 27 : 615 (1938)
12 B	<i>Lophura l. igniti</i> (Fire-backed pheasant)	Borneo (N. Y. zoo)	Coggeshall	1938	Strain 2	Am. J. Hyg. 27 : 615 (1938)
<i>P. durae</i> 13 A	<i>Melagris gallopavo</i> (Domestic turkey)	Kenya Colony, E. Africa	Herman	1941		Am. J. Hyg. 34G : 22 (1941)
Undescribed 50 A	<i>Pedioetes phasianellus</i> cum- <i>pestris</i> (Sharp-tailed grouse)	Lower Souris Refuge, N. D.	Wetmore	1939	" <i>P. pedioecetes</i> "	J. Wildlife Manage- ment 3 : 361 (1939)

e.g., 3H2-23 or whatever numeral is next available. In other words, the terminal portion of the designation (the portion following the hyphen) will not reflect obviously the genealogy of the strain. The genealogy will need to be sought from published accounts or from the committee.

The first series of designations are in Tables 1-3. The committee hopes to keep the list up to date by additions at such intervals as appear to be required.

Since the value of this system of terminology depends in large degree upon the cooperation of all malariologists working on avian malaria, the committee hopes that henceforth investigators when publishing the results of research will refer to

TABLE 3.—Sub-strains of *P. cathemerium*

Designation	Names in current usage	Derivation	Reference
3H1	Clon or "N" strain	Through single cell isolation by Stauber, at University of Chicago, 1933	J. Parasitol. 25: 95 (1939)
3H2	Mosquito passage	Obtained from University of Chicago in March, 1938, since transferred exclusively by mosquito bite by Beckman, Marquette Medical School	Am. J. Trop. Med. 21: 795 (1941)
3H3	Hartman-Hewitt; Phenylhydrazine	Virulence seemingly modified through treatment with phenylhydrazine by Hewitt at Johns Hopkins University	Am. J. Hyg. 29C: 135 (1939)
3H1-1	"U"	Stauber's clon passed through 24 blood passages, then transmitted by <i>Culex pipiens</i> by Huff at University of Chicago, Oct. 1937	J. Infect. Dis. 68: 184 (1941)
3H1-2	"P"	Stauber's clon passed through 30 blood passages, then transmitted by <i>Culex pipiens</i> by Huff at University of Chicago, Jan. 1939. Ability to produce normal numbers of gametocytes restored	J. Infect. Dis. 68: 184 (1941)
3D1	Atypical; "F"; agametocyte strain	Gametocytes lost during rapid blood passage, Sept. 1932. Huff and Gambrell, University of Chicago. Periodicity disturbed; merozoite number decreased	Am. J. Hyg. 19: 404 (1934)
3M1	None	Derived from small numbers of parasites in attempt to isolate clons. Less pathogenic for canaries than parent strain	None
3M2	None	Derived from small numbers of parasites in attempt to isolate clons. Less pathogenic for canaries than parent strain.	None

the strains used by the designations given by the committee. It is further urged that before publishing results of work on new strains or sub-strains investigators secure designations from the committee for such strains and incorporate them in their publications. It should be emphasized that the species name should be given in addition to the strain designation, e.g., "strain 3D1 of *P. cathemerium*."

COMMITTEE ON TERMINOLOGY OF STRAINS OF AVIAN MALARIA

CLAY G. HUFF, *Chairman*
GEORGE H. BOYD
REGINALD D. MANWELL

The Journal of Parasitology

Volume 28

AUGUST, 1942

Number 4

THE HELMINTHS OF RACCOONS IN EAST TEXAS

ASA C. CHANDLER

Biological Laboratory, Rice Institute, Houston, Texas

Through the kind coöperation of Mr. Rollin H. Baker, Field Biologist of the Texas Game, Fish and Oyster Commission, 13 raccoons (*Procyon lotor lotor*) from Angelina County, in East Texas, were made available for parasitological examination. Nine of the specimens were trapped during early December; the other four were obtained in May and September. All of the raccoons were heavily parasitized, containing from four to eight species of worms. One animal was estimated to contain about 50,000 *Pharyngostomoides procyonis*, one large *Mesocostoides* and several immature ones, a dozen hookworms, a few *Molineus* and several spiny-headed worms in the intestine; eight gnathostomes in the stomach; several hundred *Eurytrema* in the pancreas; and several guinea worms in each hind foot. Two kinds of parasites, gnathostomes and guinea worms, were markedly seasonal, being abundant in winter but not at other times.

Of 12 species of helminths found, 7 are new species. Notes on each of the species encountered and descriptions of the new species follow.

Pharyngostomoides procyonis Harkema, 1942

This fluke, recently described by Harkema (1942) from raccoons in North Carolina, was found in every one of the raccoons examined. Some hosts harbored only a few dozen worms, whereas others harbored tens of thousands; one case was estimated to harbor 50,000 of these flukes.

Like many DIPLOSTOMIDAE this species is extremely variable in shape and size. The size is undoubtedly in part a matter of age: eggs may begin to be produced when the worms are less than 0.5 mm in length, although at this time the vitellaria are still incompletely developed. It is also possible that the number of flukes harbored and the presence of prior infections may influence the size of the worms.

Fibricola texensis Chandler, 1942

This new species of *Fibricola* is being described elsewhere, along with

Received for publication, August 5, 1941.

a complete account of its life cycle. It was found in 6 of the 13 raccoons but never in as large numbers as the *Pharyngostomoides procyonis* which always accompanied it. Its parthenitae develop in *Physa anatina* and its diplostomula in tadpoles.

Eurytrema procyonis Denton, 1942

This new species of *Eurytrema* is being described elsewhere (Denton, 1942), along with a partial account of its life cycle. It was found in the pancreas of 7 of the 13 raccoons examined, usually in large numbers. One host contained over 1000 specimens, and two others contained over 500 each. The parthenitae of this fluke develop in the land snail, *Mesodon thyroidus*, but the second intermediate host, which is probably required, has not been determined.

Mesocestoides variabilis Mueller, 1928

Adult specimens corresponding with Mueller's description of this worm were obtained from two raccoons, and immature ones, still in the tetrathyridium stage, from three others. They are discussed in more detail elsewhere (Chandler, 1942b). Some specimens obtained from a dog and from a raccoon in Nebraska, reported by Coatney (1936), have been examined by the writer, and are believed to belong to this same species. Numerous immature specimens of a *Mesocestoides* were also reported by Morgan and Waller (1940) from a raccoon in Iowa, but it was not possible to determine the species.

Since the raccoon specimens are intermediate in size between Mueller's typical *variabilis* from *Spilogale* and *Urocyon* and his variety *maior* from *Mephitis*, there no longer seems to be justification for distinguishing these varieties.

Ochoristica procyonis n. sp.

(Plate I, Figs. 1-3)

Description: Strobila 8 to 23 mm long, consisting of about 45 to 70 proglottids; maximum width 0.9 to 1.6 mm. Scolex 300 to 375 μ across and about 150 to 200 μ long, usually demarcated from neck, which is wider, by slight constriction. In contracted specimens suckers partly withdrawn into pocket-like depressions, and anterior portion of scolex retracted to give the appearance of a rostellum, as Baer (1935) figured for *O. incisa*. Suckers small, 90 to 100 μ in diameter, in dorsal and ventral pairs facing antero-dorsad and antero-ventrad; members of each pair only 23 to 32 μ apart. Neck very short, segmentation evidenced by lateral serration within 100 to 200 μ of scolex. Margins of worm serrate entire length. Segments broader than long except near end of strobila in contracted specimens, but mature segments about square and terminal segments several times as long as broad in relaxed specimens. Mature segments 0.28 to 0.7 mm long and 0.7 to 1.05 mm wide; terminal ripe segments 1 to 2.3 mm long and 0.9 to 1.6 mm broad. Genital atrium about 70 to 90 μ deep, commonly closed by a pair of lips and with a small ante-chamber into which the sperm duct opens, connected with the atrium by a minute curved duct (Fig. 1). Genital pores irregularly alternating, $\frac{1}{3}$ to $\frac{1}{2}$ length of proglottid from anterior border. Only two pairs of excretory tubes seen, the ventral ones very thin walled and about

twice diameter of dorsal ones, which pursue a wavy course; transverse tubes not seen. Genital ducts pass dorsal to excretory canals.

Testes 48 to 63, round or transversely flattened according to state of contraction of segment, measuring 30 by 40 μ to 50 by 70 μ ; situated between excretory vessels, mostly posterior and lateral to female glands, a few anterior-lateral or occasionally anterior, almost as numerous on poral as aporal side, and for most part in single dorsal layer. Cirrus pouch 110 to 130 μ long and 40 to 45 μ wide, entering genital atrium indirectly through ante-chamber; inner end of cirrus pouch 200 to 220 μ from margin of proglottid, extending far beyond excretory canals. Vas deferens much coiled anterior to ovary on poral side.

Ovary large, much branched, roughly reniform, in anterior median portion of segment, occupying about half width of segment; maximum breadth in mature segments to 375 to 530 μ . Vitelline gland also consisting of clusters of lobes, tending to become somewhat confluent in older segments; maximum size 175 to 195 μ broad and 115 to 175 μ long. Vagina, opening into genital atrium from behind, nearer margin than cirrus, follows contour of cirrus for about half its length, then pursues its course to oötype in smooth curve dorsal to ovary. Seminal receptacle forms as enlargement of vagina dorsal to ovary and not far from oötype; it measures 60 to 80 μ long and 30 to 35 μ wide in mature segments, but increases to about 130 by 65 μ in post-mature segments. Mehlis' gland spherical, situated medially between ovary and vitelline gland, anterior to center of segment. Uterus rapidly spreads out over entire segment; ovary and vitelline glands quickly disappear, but testes, cirrus pouch, vagina and seminal receptacle persist almost to end. Onchospheres (in mounted segments) about 25 to 28 by 30 to 34 μ , with hooks 13 μ long.

Hosts: *Procyon lotor lotor*.

Location: Small intestine.

Locality: Angelina County, Texas.

Type specimen: U. S. Nat. Mus. Helm. Coll. No. 44942; Paratypes, No. 44943.

Leigh (1940) obtained 75 specimens of an *Oochoristica* from one of six raccoons in Illinois, but was unable to make a specific determination because of the immaturity of the worms. Three other species of *Oochoristica* have been reported from carnivores in the Western Hemisphere: *O. mephitis* Skinker, 1935, from *Mephitis* in Georgia; *O. oklahomensis* Peery, 1939, from *Spilogale* in Oklahoma; and *O. sandgroundi* (*Atrio-taenia parva* Sandground, 1926) Baer, 1935, from *Nasua* in Brazil. *O. procyonis* differs markedly from the two skunk species in the size and shape of the cirrus pouch, and in having irregularly alternating genital pores. It most nearly resembles *O. sandgroundi* but is a larger worm, differing in having a much smaller scolex, smaller suckers, and longer cirrus pouch. Among other mammalian forms it most nearly resembles *erinacei* in most respects, but differs in having a broader scolex, smaller suckers, genital ducts dorsal to excretory canals, presence of a seminal receptacle, and more numerous testes.

Only one raccoon harbored this worm, but in this animal several dozen specimens were present.

Macracanthorhynchus ingens (v. Linst. 1879) Meyer, 1933

This acanthocephalan was found in 11 of the 13 raccoons, in numbers from 1 to about 80 per host. An account of the life cycle of this worm,

which involves a white grub as an intermediate host, is to be reported elsewhere by Donald V. Moore.

Arthrocephalus lotoris (Schwarz, 1925) n. comb.

This hookworm was found, in moderate numbers, in all of the raccoons examined. It appears to be widely distributed in raccoons in the United States as Price (1928) surmised when he found it in a civet, *Bassariscus astutus flavus* in Texas, far from Maryland, where Schwartz first found it in raccoons. In addition to the Maryland and Texas records it is almost certain that the hookworms found by Leigh (1940) in two of six raccoons in Illinois were representatives of the same species. Leigh designated his worms "*Arthrocephalus* sp.," and called attention to their similarity to Vaz's (1935) *A. maxillaris* except in certain characters of the buccal capsule.

Schwartz (1925) described this hookworm from a raccoon in Maryland under the name *Uncinaria lotoris*. Ortlepp (1925), earlier in the same year, erected a new genus *Arthrocephalus* for a new species of hookworm, *A. gambiensis*, from an African mongoose. The most striking characteristic of this genus is the presence of sutures which divide the buccal capsule into a number of articulated cuticular plates. Schwartz' *Uncinaria lotoris* possesses these sutures and has an arrangement of plates so similar to those of the type species of the genus that there can be no question that the two are cogenetic. There are, however, some characters in which these two worms do not agree, thus necessitating an emendation of the genus. Ortlepp included as characters of the genus, besides the completely articulated buccal capsule, the absence of buccal lancets, the presence of a well developed dorsal cone, and the longitudinal arrangement, without transverse coils, of the loops of the female genitalia. In none of these characters does *A. lotoris* agree with *A. gambiensis*, for the former species possesses a pair of obtuse ventral lancets, lacks a dorsal cone, and has transverse coils in the female genitalia. In all these respects it more nearly resembles members of the genus *Uncinaria*, in which Schwartz placed it, but in the writer's opinion the articulated character of the buccal capsule, shared by these two species, is sufficient reason for retaining Ortlepp's genus. The absence of ventral lancets, presence of a dorsal cone, and longitudinal arrangement of female genital organs should be dropped from the characterization of the genus.

It is possible that hookworms from *Procyon cancrivorus* in Brazil described by Vaz (1935) under the name *Arthrocephalus maxillaris* (Molin, 1860) are identical with *A. lotoris*, but Vaz's description and figures of the buccal capsule do not exactly correspond to the condition in *A. lotoris*. Vaz specifically states that no ventral lancets are present, though they are unmistakably present in *lotoris*. Although Vaz at-

tempted to identify his species with one of two briefly described by Molin in 1860 from the same host and locality, Molin's descriptions are so incomplete that, as Schwartz pointed out, they are unrecognizable. If Vaz's form from *Procyon cancrivorus* should prove to be identical with the North American form, his name *maxillaris* should be considered a synonym of *lotoris*.

Molineus barbatus n. sp.

(Plate I, Figs. 4-6)

Description: Extremely delicate red worms with finely striated cuticle and with 24 to 28 longitudinal ridges. Cephalic extremity with a coarsely striated cuticular enlargement 50-55 μ long, not greatly inflated. Diameter of head 25 to 29 μ . Annular sulcus at level of excretory pore well marked, 190 to 200 μ from anterior end. Cervical papillae not observed. Nerve ring just anterior to excretory pore. Esophagus clavate, 360 μ long in males, 420 to 480 μ long in females, with maximum diameter of 35 to 40 μ .

Females 5.5 to 6.6 mm long; maximum diameter 68 to 74 μ . Vulva salient, 0.9 to 1 mm from posterior end, dividing body about 5:1. Anterior ovejector about 0.9 mm long, containing 9 to 12 eggs; posterior one about 0.7 mm long containing 6 to 9 eggs. Eggs elliptical, 50 to 53 μ by 32 to 37 μ . Anus 56 to 61 μ from posterior end; tail bluntly trilobed at tip, 15 μ broad, the middle lobe bearing a very slender spine 13 to 18 μ long.

Males 4.3 to 4.7 mm long with maximum diameter of 60 to 64 μ . Bursa with large lateral lobes and medium-sized, clearly-demarcated dorsal lobe. Inner surface of lateral lobes between lateral and ventral rays studded with numerous well-defined spines, thinning out ventral to ventral rays. Ventral rays coarser than lateral, diverging only at tips, and reaching margin of bursa. Antero-lateral ray shorter, diverging from other lateral rays but remaining much nearer to them than to ventrals. Medio-lateral and postero-lateral rays contiguous, long and slender, reaching margin of bursa. Externo-dorsals thicker than any other rays, curving away from dorsal to become almost contiguous with lateral rays. Dorsal bifurcated distally, each branch again bifurcated, the inner of the terminal branches ending in two prongs, the other one single, thus giving appearance of three short prongs at end of each primary bifurcation. Spicules equal, 90 to 100 μ long, with a conspicuous medially-directed barb anteriorly, and ending in two pointed processes, outer one of which may represent two closely applied processes; inner process smaller and shorter than outer one. Gubernaculum slender, 50 to 55 μ long and only 7 μ wide.

Host: *Procyon lotor lotor*.

Location: Small intestine.

Locality: Angelina County, Texas.

Cotypes (δ and η): U. S. Nat. Mus. Helm. Coll. No. 44930.

This species is the second of its genus to be reported from North America. Skinker, 1932, reported *M. patens*, of Old World MUSTELIDAE, from *Canis lestes* in Washington and from a mink in Mississippi. Sprehn (1932), however, described another species, *M. americanus*, from an American mink in Europe. Cameron (1936) obtained a single decomposed female *Molineus* from *Procyon cancrivorus* in Trinidad, but suspected that it belonged to the species *M. barbaris*, which he found in *Tayra barbarus* in Trinidad. The species here described is relatively more slender than any heretofore reported. It is much smaller than *M. americanus*, with spicules only half as long, and shaped differently. It

is also much smaller than *M. patens* and has much smaller and more slender spicules. The form of the spicules, arrangement of the dorsal rays, and large number of longitudinal cuticular ridges are sufficient to distinguish it from any other species so far described. This worm was obtained in small numbers from 4 of the 13 raccoons examined.

Synhimantus longigutturata n. sp.

(Plate I, Figs. 7-8)

Description: Female 3.54 mm long, with maximum diameter of 65 μ ; diameter at origin of esophagus 35 μ . Two prominent lips about 8 μ long and 7 μ wide dorso-ventrally. Bottom of dorso-lateral and ventro-lateral loops of cephalic cordons 65 μ from anterior end; top of recurrent lateral loops 38 μ from anterior end. Excretory pore 230 μ from anterior end. Pharynx extending far posterior to end of cordons, to 145 μ from anterior end. Esophagus 265 μ long, in two parts; anterior glandular part 155 μ long. Vulva 1.5 mm from posterior end, dividing body about 4:3. Anus 96 μ from posterior end. Tail has bluntly rounded end, 9 μ in diameter. Eggs in oviducts about 40 by 24 μ . Male unknown.

Host: *Procyon lotor lotor*.

Location: Small intestine.

Locality: Angelina County, Texas.

Type specimen: U. S. Nat. Mus. Helm. Coll. No. 44928.

Since all known members of this and related genera of the family ACUARIIDAE are parasites of the anterior portion of the alimentary canal of birds, it is probable that the single female worm found in a raccoon had been ingested with a bird host. The finding is of interest, however, since there is only one previous record of a member of this genus in North America ("*Dispharagus egrettae* Rud. 1819" of MacCallum, 1921, = *Synhimantus invaginata* von Linst. 1901; *vide* Vigueras, 1936). The worm differs from all previously described species of the genus in its minute size and in the relatively extreme length of the buccal vestibule, which is nearly half the length of the esophagus and extends far beyond the posterior extremities of the cordon loops.

Gnathostoma procyonis n. sp.

(Plate II, Figs. 12-15)

Description: Large stout worms, tapering only slightly at the ends. Head bulb with 9 or 10 rows of spines, 90 to 100 spines in each row, set about 22 μ apart; protruding portions of hooks about 15 μ long. Body clothed with scales for approximately half its length, the scales then running together to form serrated ridges or striations around body. Posterior portion of body marked by coarse transverse wrinkles independent of the serrated striations, 50 to 100 μ apart, giving cuticle in this region a corrugated appearance. Cuticle in posterior portion of body also markedly inflated, giving body an irregular bloated appearance. Scales very large, with only three, occasionally four, principal points, but usually with one or several minor points or serrations. Immediately behind head bulb bases of scales about 20 μ broad, set about 50 μ apart, and having short blades (Fig. 13a). After first few rows bases of scales become somewhat smaller, about 15 μ broad, set about 25 to 28 μ apart, the blades becoming longer and broadening out, assuming shape of an elongated maple leaf (Figs. 13b and c). Most of scales have a long middle point and two shorter lateral points, usually with one or more minor serrations. Occa-

sionally one of minor serrations becomes large enough to make scale 4-pointed instead of 3-pointed. Throughout most of scaled area scales about 20μ apart. Scales reach a maximum length of 80 to 85μ with width of 20 to 25μ . Near middle of body a rather sudden transition from rows of separate scales to serrated ridges; scales first have their points reduced to short terminal prongs, then become shortened, and finally become fused at base into continuous serrated ridges (Figs. 13e and f). Esophagus club-shaped, about one-fifth length of body. Cervical sacs 1.6 to 1.85 mm long, measured from posterior border of head bulb.

Female, 20 to 26 mm long, with a maximum diameter, where cuticle is not inflated, of about 1.1 to 1.2 mm, but where inflated in posterior portion of body may reach 1.5 to 1.6 mm. Head bulb about 700 to 800μ broad and 350 to 370μ long. Esophagus 4.2 to 4.3 mm long. Vulva 8.25 to 8.5 mm from posterior end, dividing body $2:1$ or $3:2$. Ovejector (Fig. 14), provided with three nearly spherical glands near its exit, turns forward in body, remaining narrow (240μ in diameter) with thick muscular walls for a distance of about 2.8 mm. Here it widens out into a thin walled tube filled with eggs, 560μ in diameter. It continues forward, narrowing to about 200μ , and bending backward about 8 to 9 mm anterior to vulva. In some specimens it bifurcates into two posteriorly directed uteri just behind the turn, in others 2 or 3 mm behind this point. Anus about 280μ from posterior end, which is bluntly rounded and provided with usual three papillae, the lateral ones of which, as seen in ventral view, are much broader than the median one (Fig. 12a). Extreme width across lateral papillae about 210μ .

Male, 16 to 19 mm long, with a maximum diameter of about 800μ where cuticle is not inflated, but expanding to 1.15 mm where inflated in posterior half of body. Head bulb 650 to 700μ broad and 280 to 325μ long. Esophagus 3.4 to 3.5 mm long. Anus 360 to 380μ from posterior end, the bursa-like expansion about 850 to 900μ in diameter. The four pairs of large lateral papillae lie close together (Fig. 15), the posterior pair slightly shorter but not more slender than the others, and not markedly separated from them. Under surface of bursa with fine spines, only slightly larger than those on posterior body striae, only on portion anterior to papillae; rest of bursa unspined. No ventral papillae seen. Left spicule short, 550 to 720μ long, about 35μ broad at the base, 25μ broad in its basal half, then abruptly narrowed to about 12μ in distal portion. Long right spicule very variable in length, 1.8 to 2.95 mm, 85μ broad at its base, 55μ broad in its proximal portion, narrowing to about 26μ distally.

Host: *Procyon lotor lotor*.

Location: Stomach.

Locality: Angelina County, Texas.

Cotype specimens: U. S. Nat. Mus. Helm. Coll. No. 44931.

This species resembles *G. spinigerum* more closely than it does *G. didelphis*. It is smaller than *didelphis* and differs in the size and shape of the body spines; in the manner of termination of the spines, which in *procyonis*, as in *didelphis*, goes over to serrated ridges, while in *spinigerum* the spines become small but remain separate and distinct; in the inflation of the cuticle; in the size and shape of the papillae at the posterior end of the body of the female; in the shape and relative lengths of the spicules; in the lack of spination of the bursa; and in the even spacing of the lateral caudal papillae of the male. It differs from *spinigerum* in the shape of the body scales; in the transition to serrated ridges instead of separate, widely-spaced spines; in the shape of the papillae at the posterior end of the body of the female; in the lack of spination of the bursa; and in the shape and relative size of the most posterior lateral papillae of the male.

This worm was found in the stomachs of all 9 of the raccoons examined in December, in numbers from 1 to 58 per host. In one raccoon examined in May a single adult was found in the stomach, although there were scars where others had been. In 3 animals examined in September no gnathostomes were found. It seems evident that this species is of markedly seasonal occurrence. When present in numbers these are undoubtedly the most harmful parasites harbored by raccoons. The stomach wall in a few specimens was veritably riddled by them.

In the raccoon examined in May a larval female gnathostome was found encysted in the skin. It resembles those described by the writer (1925) from the liver of cats experimentally infected with cysts of *G. spinigerum* from snakes. It is 6.5 mm long and 500 μ in diameter, with the four rows of spines on the head bulb characteristic of fourth-stage gnathostome larvae. The spiny annulations of the body fade out at or slightly before the middle of the body (at the junction of the second and last thirds in *spinigerum* larvae), and the vulva is situated 72% of the body length from the anterior end (60–65% in *spinigerum* larvae). Whether this represents an aberrant larva of *G. procyonis*, or of *G. didelphis* in a foreign host, cannot be determined until larvae of both these species have been found and compared.

Dirofilaria tenuis n. sp.

(Plate I, Figs. 9–11)

Description: Females (males unknown) slender worms 8.5 to 9.6 cm long, with maximum diameter of 310 to 330 μ . Caudal end bent dorsad. Cuticle rather conspicuously marked by transverse striations about 5 μ apart, and longitudinal ridges, more or less broken and branched (Fig. 10), about 10 μ apart. Esophagus 1.12 to 1.4 mm long, and 50 μ in diameter. Nerve ring 275 to 300 μ from anterior end. Cervical papillae small, about 430 μ from anterior end. Vulva almost immediately behind end of esophagus, 1.23 to 1.5 mm from anterior end. Vagina directed posteriorly, 4.5 to 5 mm long, then dividing into two opisthodelphous uteri. Anus difficult to see, 125 to 165 μ from posterior end. Tail bluntly rounded; diameter at anus 117–130 μ . Microfilariae in blood 220 to 250 μ long and 4 to 5 μ in diameter, with finely striated cuticle. Anterior end bluntly rounded, first 9 to 10 μ without nuclei. Tail very finely drawn out. Nerve ring 20 to 24% of length from anterior end; excretory pore 35%; genital cell 60%; anal break 80%; last nucleus 90%.

Host: *Procyon lotor lotor*.

Location: Subcutaneous tissue of belly and flanks.

Locality: Angelina County, Texas.

Type specimen: U. S. Nat. Mus. Helm. Coll. No. 44929; paratype (microfilariae) No. 44940.

This *Dirofilaria*, belonging to the skin-inhabiting division of its genus for which Faust (1937) erected the subgenus *Nochtiella*, differs from others of this group in its slenderness, the distance of the anus from the posterior end, and the cuticular markings.

Several other forms of subcutaneous *Dirofilaria* have been reported from North America. *D. scapiceps* from rabbits is a relatively short,

stout worm with short spicules. Three species (*spinosa*, *subcutanea*, and *repens*) were reported from porcupines by Canavan (1921, 1923), but Lent and Freitas (1937) consider these all alike; they are all larger and stouter worms than *D. tenuis*. Chitwood (1933b) examined worms from a Canadian lynx which he referred to the species *acutiuscula*, expressing the opinion, without giving reasons, that *repens* is identical with *acutiuscula*, and therefore a synonym. Some parasitologists (Faust, 1937; Lent and Freitas, 1937) have not been willing to accept Chitwood's dictum. The members of this genus are notoriously variable in size and proportions and in details of spicules and male caudal papillae, so differentiation of species is difficult. Either most of the species must be merged for lack of clear-cut morphological characters which do not overlap, or one must provisionally accept forms from different types of hosts as specifically distinct until more is known about their variations and biology. It is improbable that *acutiuscula*, found in the stomach walls of peccaries in South America, is identical with *repens*, found subcutaneously in dogs in Europe, especially when only a single case has been recorded of a subcutaneous *Dirofilaria* in South American dogs.

The cuticular markings of most species of *Dirofilaria* have not been carefully described. Fülleborn (1908) gave a figure of the cuticle of *repens* showing very fine longitudinal striations in addition to the transverse ones. Lent and Freitas (1937) stated that *D. acutiuscula* has about 70 longitudinal ridges which, according to their figure, are 16 to 24 μ apart. In *tenuis* there are about 90 ridges, conspicuously broken and branched, and spaced only about 10 μ apart. *D. immitis* shows no longitudinal ridges at all, and much less conspicuous transverse ridges. The fact that in the descriptions of other species of *Dirofilaria* the longitudinal ridges are not mentioned suggests that they must be much less conspicuous than in *tenuis*. It is possible that these markings may provide reliable specific characters in this genus.

Only one of the raccoons examined yielded specimens of this worm, and none of the others contained microfilariae in the blood. Only three female specimens were found, coiled in the subcutaneous tissue of the belly and flanks. A careful search for males was fruitless.

Dracunculus insignis (Leidy, 1858) n. comb.

A guinea worm very closely resembling the species attacking man in the Old World, *Dracunculus medinensis*, except in size, was found in 6 of the 9 raccoons examined in December, but not in any of 6 (2 not autopsied) examined in May and September. In one case living worms were found in both front and hind feet, and in one case there were dead calcified worms in a front foot and living ones in the hind feet, but in all the other cases the worms were found only in the subcutaneous tissue of one or

both hind feet, much twisted and coiled, and occupying little space for their size. The worms varied from 7.6 to 28 cm in length, with a diameter of 0.84 to 1.08 mm. Specimens with embryos were 20 to 28 cm long. In only one of the nine raccoons examined in the first half of December had an ulcer formed for the escape of the embryos. Trappers in East Texas say that such ulcers are frequently seen in January and February.

This is the first time that guinea worms have been found with regularity in any host in the United States, although a number of sporadic reports are available (Benbrook, 1932, in a fox in Iowa; Chitwood, 1933a, in raccoons in Ontario and New York and mink in Nebraska; and Benbrook, 1940, in a dog and mink in Iowa). Chitwood called attention to the probability that Leidy's (1858) *Filaria insignis* from a raccoon was also a guinea worm. Unfortunately Leidy's specimen appears now to be missing, but there seems little reasonable doubt that Leidy was describing the same worm that is here reported and that his name is acceptable for the species providing it is not identical with *D. medinensis*.

Chitwood was of the opinion that the worms from American wild carnivores are specifically identical with *Dracunculus medinensis* in spite of their much smaller size. The writer, however, is of the opinion that the cephalic structures of the North American worm do not correspond exactly with those of *D. medinensis* as described and figured by Moorthy, though they closely resemble those figured by Travassos (1934) for *D. fuelleborni* from an opossum in South America. Good specific characters are notably few in the females of this genus, and the writer, on epidemiological grounds, considers it very doubtful that *insignis* and *medinensis* are identical. It is hoped that in another season the males can be obtained from experimental infections in dogs and that these may provide definite means of identification. A fuller account of these guinea worms, with a report of a possible human case, and a discussion of the epidemiological grounds for considering them distinct from *D. medinensis*, are being published elsewhere (Chandler, 1942c).

SUMMARY

An examination of 13 raccoons from East Texas revealed the following parasites in the incidences given: *Pharyngostomoides procyonis*, 13 cases; *Fibricola texensis*, 6 cases; *Eurytrema procyonis*, 7 cases; *Mesocestoides variabilis*, adults, 2 cases, tetrathyridea, 3 cases; *Oochoristica procyonis* n. sp., 1 case; *Macracanthorhynchus ingens*, 11 cases; *Arthrocephalus lotoris*, 13 cases; *Molineus barbatus* n. sp., 5 cases; *Synhimantus longigutturata* n. sp. (probably pseudoparasitic), 1 specimen; *Gnathostoma procyonis* n. sp., 11 cases; *Dirofilaria tenuis* n. sp., 1 case; *Dracunculus insignis*, 6 cases. Each raccoon harbored from 4 to 8 species

of helminths. *Pharyngostomoides procyonis* was the most abundant parasite, *Gnathostoma procyonis* the most harmful.

BIBLIOGRAPHY

- BAER, J. G. 1927 Monographie des cestodes de la famille des Anoplocephalidae. Bull. Biol. France et Belgique Suppl. 10: 1-241.
- BENBROOK, E. A. N. 1932 *Dracunculus medinensis* (Linnaeus, 1758) appears in the United States as a parasite of the fox. J. Am. Vet. Med. Assn. 81: 821-824.
- 1940 The occurrence of the guinea worm, *Dracunculus medinensis*, in a dog and in a mink, with a review of this parasitism. J. Am. Vet. Med. Assn. 96: 260-263.
- CAMERON, T. W. M. 1936 Studies on the endoparasitic fauna of Trinidad. III. Some parasites of Trinidad carnivores. Canad. J. Research, 14D: 25-38.
- CANAVAN, W. P. N. 1929 Nematode parasites of vertebrates in the Philadelphia Zoological garden and vicinity. I. Parasitology 21: 63-102.
- 1931 Ibid. II. Parasitology 23: 196-229.
- CHANDLER, A. C. 1925 A contribution to the life history of a gnathostome. Parasitology 17: 237-244.
- 1932 Notes on the helminth parasites of the opossum. (*Didelphis virginiana*) in Southeast Texas, with descriptions of four new species. Proc. U. S. Nat. Mus. 81 (Art. 16): 1-15.
- 1942a The morphology and life cycle of a new strigeid, *Fibricola texensis*, parasitic in raccoons. Trans. Am. Micr. Soc. 61: 156-167.
- 1942b *Mesocestoides manteri* n. sp. from a lynx, with notes on other North American species of *Mesocestoides*. J. Parasitol. 28: 227-231.
- 1942c The guinea worm, *Dracunculus insignis* (Leidy, 1858), a common parasite of raccoons in East Texas. Am. J. Trop. Med. 22: 153-157.
- CHITWOOD, B. G. 1933a Does the guinea worm occur in North America? J. Am. Med. Assn. 100: 802-804.
- 1933b Note on a genus and species of nematode from *Lynx canadensis*. J. Parasitol. 20: 63.
- COATNEY, G. R. 1936 Some notes on cestodes from Nebraska. J. Parasitol. 22: 409.
- DENTON, FRED 1942 *Eurytrema procyonis* n. sp. (Trematoda: Dicrocoelidae) from the raccoon, *Procyon lotor*. Proc. Helm. Soc. Washington 9: 29-30.
- DUBOIS, G. 1938 Monographie des Strigeida (Trematoda). Mém. Soc. Neuchâteloise Sc. Nat. 6: 1-535.
- FAUST, E. C. 1937 Mammalian heart worms of the genus *Dirofilaria*. Festsch. Nocht., pp. 131-139.
- FÜLLEBORN, F. 1908 Ueber Versuche an Hundefilarien und deren Uebertragung durch Mücken. Arch. Schiffs- u. Tropen-Hyg. 12: 1-43.
- HARKEMA, R. 1942 *Pharyngostomoides procyonis* n. g., n. sp. (Strigeida), a trematode from the raccoon in North Carolina and Texas. J. Parasitol. 28: 117-122.
- HUGHES, R. C. 1940 The genus *Oochoristica* Luhe, 1898. Am. Midland Naturalist 23: 368-381.
- LEIDY, J. 1858 Contributions to helminthology. Proc. Acad. Nat. Sc. Phila. 10: 110-112.
- LEIGH, W. H. 1940 Preliminary studies on parasites of upland game birds and fur-bearing mammals in Illinois. Ill. Nat. Hist. Bull. 21: 185-194.
- LENT, H. AND DE FREITAS, J. F. T. 1937 Contribuição ao estudo do genero *Dirofilaria* Railliet and Henry, 1911. Mem. Inst. Oswaldo Cruz 32: 37-51.
- MORGAN, B. B. AND WALLER, E. F. 1940 Severe parasitism in a raccoon (*Procyon lotor lotor* Linnaeus). Tr. Am. Micr. Soc. 59: 523-527.

- ORTLEPP, R. J. 1925 On *Arthrocephalus gambiensis* n. g., n. sp., a new ankylostome from an African mongoose. J. Helm. 3: 151-156.
- PEERY, H. J. 1939 A new unarmed tapeworm from the spotted skunk. J. Parasitol. 25: 487-490.
- PRICE, W. W. 1928 The civet, *Bassariscus astutus flavus*, a new host for *Uncinaria stenocephala*. J. Parasitol. 14: 197.
- RAILLIET, A., AND HENRY, A. 1911 Sur une filaire peritoneale des porcins. Bull. Soc. Path. Exot. 4: 386-389.
- SANDGROUND, J. H. 1926 A new mammalian cestode from Brazil. In "Medical report of the Hamilton Rice expedition to the Amazon, in conjunction with the department of tropical medicine of Harvard University, 1924-1925," Harvard Univ. Press, pp. 285-291.
- SCHWARTZ, B. 1925 A new species of hookworm from a North American raccoon. Proc. U. S. Nat. Mus. 67, Art. 26: 1-4.
- SKINKER, M. S. 1932 *Molineus patens* (Dujardin, 1845) Skrjabin and Schulz, 1926, collected in the United States. J. Parasitol. 19: 91.
- 1935 A new species of *Oochoristica* from a skunk. J. Parasitol. 25: 487-490.
- SPREHN, C. 1932 Lehrbuch der Helminthologie. Berlin. 998 pp.
- TRAVASSOS, L. 1934 *Dracunculus fuelleborni* n. sp., parasito de *Didelphis aurita* Wied. Mem. Inst. Oswaldo Cruz 28: 235-237.
- 1937 Revisão da família Trichostrongylidae Leiper, 1912. Monogr. Inst. Oswaldo Cruz 1: 1-512 pp.
- VAZ, Z. 1935 Rediscricão de *Arthrocephalus maxillaris* (Molin 1860) necatorineo parasita de *Procyon cancrivorus*. Rev. Biol. e Hyg., São Paulo 6: 9-12.
- VIGUERAS, I. P. 1936 Notas sobre la fauna parasitologica de Cuba (Parte 1: Vermes). Mem. Soc. Cubana Hist. Nat. 10: 53-86.

EXPLANATION OF PLATES

PLATE I, p. 267

- FIG. 1. *Oochoristica procyonis*. Genital atrium and cirrus pouch region; note lips guarding atrium, small ante-chamber into which cirrus pouch opens, and topographic relations of male and female openings.
- FIG. 2. *O. procyonis*. Scolex in partially retracted state.
- FIG. 3. *O. procyonis*. Mature proglottid.
- FIG. 4. *Molineus barbatus*. Anterior end.
- FIG. 5. *Molineus barbatus*. Spicules and gubernaculum.
- FIG. 6. *Molineus barbatus*. Posterior end of male.
- FIG. 7. *Synhimantus longigutturata*. Head, lateral view; Fig. 8. Same, dorsal view.
- FIG. 9. *Dirofilaria tenuis*. Anterior end of female.
- FIG. 10. *Dirofilaria tenuis*. A region near middle of body showing longitudinal cuticular markings.
- FIG. 11. *Dirofilaria tenuis*. Posterior end of female.

PLATE II, p. 268

- FIG. 12. Posterior end of females of three species of *Gnathostoma*; a, *G. procyonis*; b, *G. spinigerum*; c, *G. didelphis*.
- FIG. 13. Body scales of *Gnathostoma*. Figs. a-f, *G. procyonis*; a, first row behind head bulb; b, about 1 mm behind head bulb; c, about 5 mm behind head bulb; d, about 10 mm behind head bulb; e, about 12 mm behind head bulb; f, about 12.5 mm behind head bulb. Fig. g, *G. spinigerum*, irregularly spaced spines in transition region of body, corresponding to Fig. e or f of *G. procyonis*.
- FIG. 14. Vagina and beginning of uteri of *Gnathostoma procyonis*.
- FIG. 15. Posterior end of small male of *G. procyonis*.

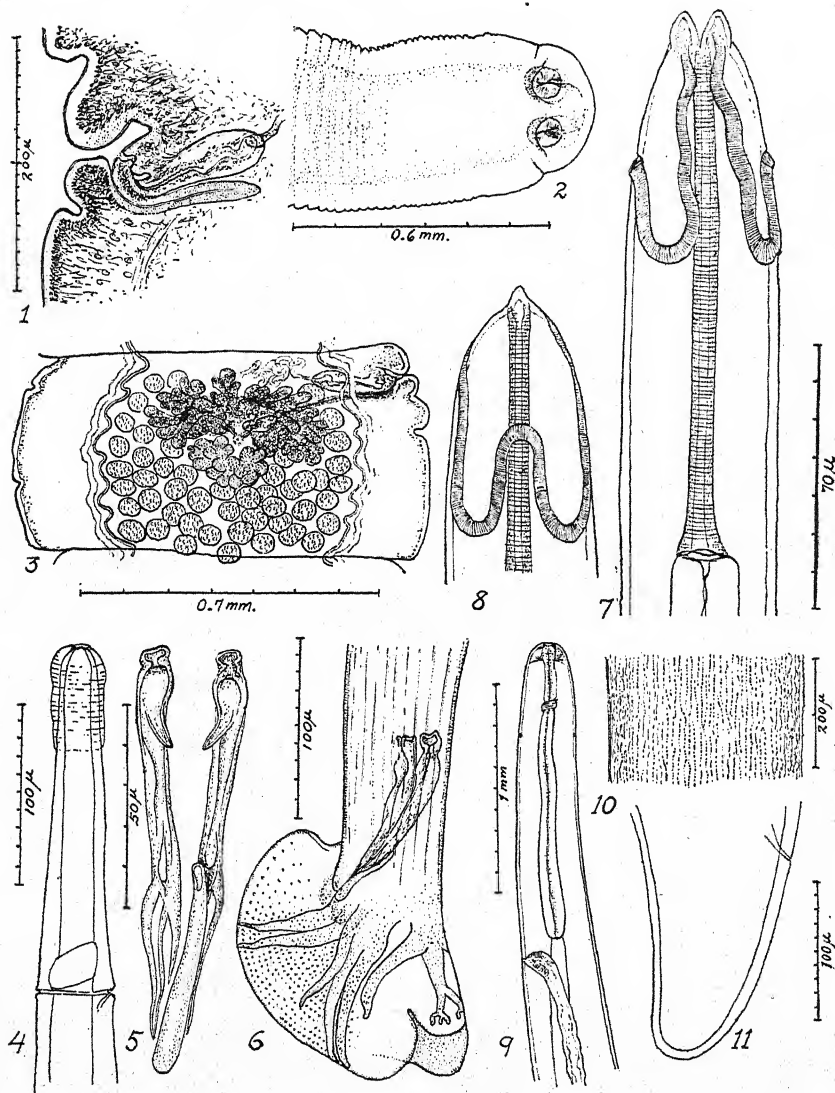


PLATE I

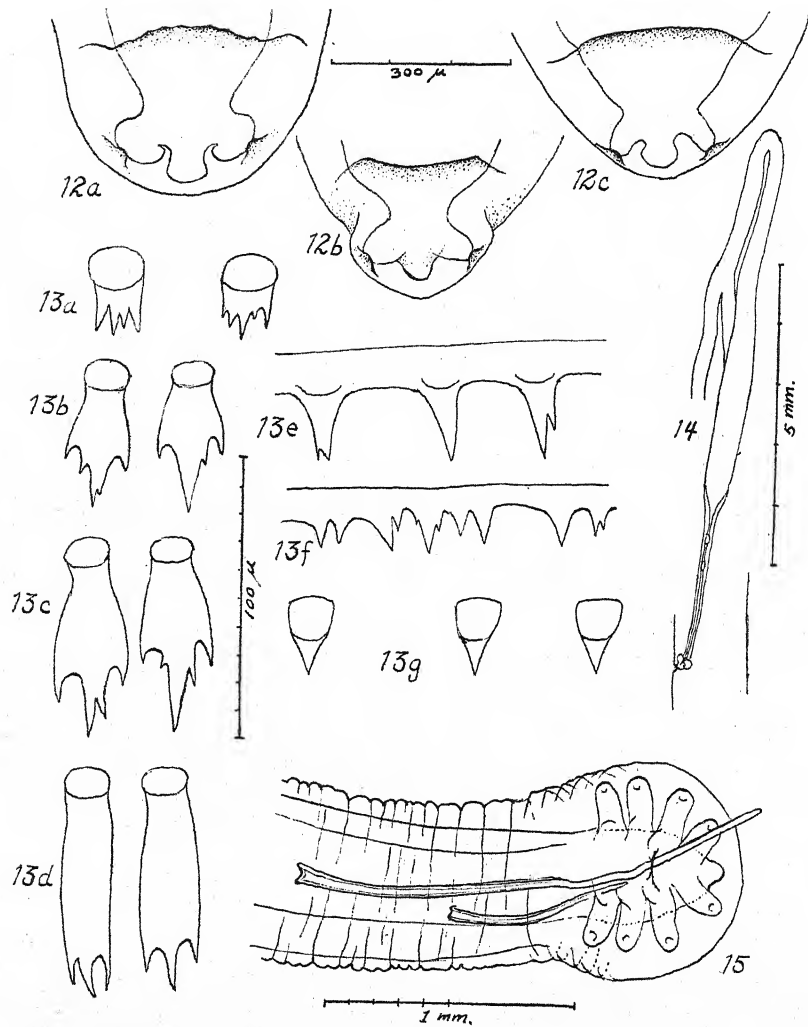


PLATE II

THREE NEW SPECIES OF *PHYLLODISTOMUM* (TREMATODA: GORGODERIDAE) FROM MICHIGAN FISHES¹

JACOB H. FISCHTHAL

Examination of the fishes of Honey Creek near Ann Arbor, Michigan, during the spring of 1941 for trematodes of the genus *Phyllodistomum* Braun, 1899, revealed the presence of three new species occurring in the ureters and urinary bladders of the northern creek chub, *Semotilus atromaculatus atromaculatus* (Mitchill), the Mississippi Valley common shiner, *Notropis cornutus chrysocephalus* (Rafinesque), and the horny-headed chub, *Nocomis biguttatus* (Kirtland). These are described as follows:

Phyllodistomum semotili n. sp.

(Fig. 1)

Description: Phyllodistomum. Body small, spatulate, consisting of a circular, discoidal posterior portion set off at middle of acetabulum from a narrower, tapering anterior portion. Cuticula spineless, bearing sensory papillae on suckers and on surfaces and margins of body. Posterior end of body slightly notched. Oral sucker subterminal, smaller than acetabulum; diameter of oral sucker to acetabulum as 1:1.5. Mouth subterminal, ventral; esophagus weakly muscular, slender, narrow; intestinal crura inflated, extending nearly to posterior end of body. Vitellaria oval, smooth, near and postero-lateral to acetabulum. Vitelline ducts joined mesially; common vitelline duct short, slightly swollen.

Ovary compact, smaller than either testis, slightly and irregularly lobate, usually in contact with vitellarium, amphitypic. Oviduct short, arising from dorsum of ovary, passing antero-mesially to oötype. Mehlis' gland weakly developed, cells small. Laurer's canal arising from oviduct just before oötype and passing antero-laterally to opening on dorsal surface. Uterus fairly extensive, coils intra- and extracecal, extending to margins of body posterior to vitellaria; uterus ascending dorsal to acetabulum, constricting to weakly muscular metraterm; latter ventral to seminal vesicle; genital atrium small. Eggs fairly numerous; older intrauterine eggs containing fully developed miracidia; latter possessing two flame cells; hatching occurring as soon as eggs enter water.

Testes oblique, fairly close together, irregularly lobate, indentations deeper and lobes more numerous than in ovary. Anterior testis usually in contact with vitellarium and opposite or slightly oblique to ovary. Posterior testis directly behind but never in contact with ovary. Vasa efferentia arising from dorsum of testes, passing forward dorsal to acetabulum to their junction slightly anterior to acetabulum; vas deferens short; seminal vesicle large; ejaculatory duct short. Genital pore mid-ventral, immediately posterior to intestinal bifurcation. Excretory pore postero-dorsal; bladder a slender, median tube extending anteriorly to level of ovary where right and left primary collecting ducts enter; latter extending anteriorly to intestinal bifurcation, then looping posteriorly a short distance each receiving two secondary collecting ducts.

Average measurements in millimeters (with maximum lengths and widths) of

Received for publication, September 8, 1941.

¹ Contribution from the Department of Zoölogy, University of Michigan.

10 specimens fixed in Bouin's fluid, stained in Mayer's paracarmine and mounted in clarite X:² Body, length 1.481 (1.275–1.620), width at intestinal bifurcation 0.268 (0.195–0.30), at anterior testis 0.608 (0.495–0.735); oral sucker, diameter 0.188 (0.150–0.203); acetabulum, diameter 0.284 (0.225–0.30); esophagus, length 0.148 (0.120–0.195); left vitellarium, length (short axis) 0.065 (0.059–0.072), width (long axis) 0.124 (0.095–0.152); right vitellarium, 0.063 (0.053–0.075) × 0.130 (0.107–0.150); ovary, 0.139 (0.120–0.158) × 0.154 (0.130–0.195); older embryonated intra-uterine eggs, 0.040 (0.037–0.043) × 0.027 (0.024–0.028); anterior testis, 0.246 (0.225–0.285) × 0.205 (0.150–0.255); posterior testis, 0.250 (0.225–0.285) × 0.218 (0.150–0.270).

Host: *Semotilus atromaculatus atromaculatus* (Mitchill).

Habitats: Ureters and urinary bladder.

Locality: Honey Creek near Ann Arbor, Washtenaw County, Michigan.

Types: U. S. Nat. Mus. Helm. Coll. No. 44947 (3 slides, type and 2 paratypes); other paratypes in author's collection.

Of 43 northern creek chubs, 8 (approximately 18.6 per cent) were infected with mature *P. semotili*. As many as 60 adult worms have been recovered from the ureters and urinary bladder of a single host.

Of 57 *P. semotili* examined, 24 had the ovary on the left and 33 had it on the right, indicating that amphitypy occurs frequently in this species.

Comparison with other species of the genus: To date 39 species of *Phyllodistomum* have been described. It is not within the scope of this paper to discuss the validity of these species, since Nybelin (1926), Holl (1929), Lewis (1935), Wu (1938) and others have already done so in their papers. The comparisons of *P. semotili* and the two new species following with the species described through 1930 are based largely upon data tabulated by Lewis (1935); all comparative data beyond his work are based upon information from the original descriptions.

Smooth vitellaria differentiate *semotili* from *acceptum* Looss, 1901, *americanum* Osborn, 1903, *angulatum* v. Linstow, 1907, *brevicicum* Steen, 1938, *carolini* Holl, 1929, *caudatum* Steelman, 1938, *conostomum* (Olsson, 1876), *elongatum* Nybelin, 1926, *entercolpium* Holl, 1930, *fausti* Pearse, 1924, *folium* (v. Olfers, 1816), *hunteri* (Arnold, 1934), *lacustri* (Loewen, 1929), *linguale* Odhner, 1902, *lysteri* Miller, 1940, *mogurndae* Yamaguti, 1934, *parasiluri* Yamaguti, 1934, *patellare* (Sturges, 1897), *pearsei* Holl, 1929, *pseudofolium* Nybelin, 1926, *sinense* Wu, 1937, *singulare* Lynch, 1936, *spatula* Odhner, 1902, *spatulaeforme* Odhner, 1902, *staffordi* Pearse, 1924, *superbum* Stafford, 1904 and *undulans* Steen, 1938.

It differs from *lesteri* Wu, 1938, *shandrai* Bhalarao, 1937 and *unicum* Odhner, 1902 in having a slightly and irregularly lobate ovary, and from *marinum* Layman, 1930, *solidum* Rankin, 1937 and *spatula* in having both ovary and testes irregularly lobate. An oral sucker smaller than the acetabulum distinguishes it further from *brevicicum*, *caudatum*, *mogurndae*, *pearsei* and *unicum*.

The extension of extrauterine coils as far forward as the vitellaria

² Clarite X was originally described as Nevillite no. 1 by Groat (1939).

and laterally to the extreme margins distinguishes it from *almorii* Pande, 1937 and *macrobrachicola* Yamaguti, 1934, and also from *americanum*, *angulatum*, *caudatum*, *conostomum*, *elongatum*, *folium*, *lacustri*, *lesteri*, *linguale*, *lysteri*, *marinum*, *pseudofolium*, *spatula*, *spatulaeforme*, *staffordi* and *unicum*.

The presence of a slight posterior notch differentiates it from *kajika* (Ozaki, 1926) and *lohrenzi* (Loewen, 1935), and also from *acceptum*, *almorii*, *brevicicum*, *carolini*, *caudatum*, *conostomum*, *entercolpium*, *fausti*, *hunteri*, *lacustri*, *lesteri*, *linguale*, *parasiluri*, *patellare*, *pearsei*, *shandrai*, *sinense*, *singulare*, *solidum*, *staffordi*, *undulans* and *unicum*.

Vitellaria that are from one-half to four-fifths the size of the ovary distinguish it from *megalorchis* Nybelin, 1926, and *simile* Nybelin, 1926, and also from *acceptum*, *americanum*, *conostomum*, *shandrai*, *spatula* and *superbum*. It differs from *macrocotyle* (Lühe, 1909) in that the testes and eggs are significantly smaller.

A significantly smaller ovary distinguishes it from all other species with the exception of *angulatum*, *brevicicum*, *carolini*, *elongatum*, *hunteri*, *macrobrachicola*, *marinum*, *pearsei* and *pseudofolium*. An ovary significantly smaller than either testis differentiates it further from *brevicicum*, *macrobrachicola*, *mogurndae*, *pearsei*, *solidum*, *undulans* and *unicum*.

Its significantly smaller body differentiates it from all other species with the exception of *carolini*, *elongatum*, *lacustri* and *pearsei*. Its host, a fish, distinguishes it further from *almorii*, *americanum*, *entercolpium*, *kajika*, *lesteri*, *patellare*, *shandrai*, *singulare* and *solidum*.

P. semotili most closely resembles *almorii*, *carolini*, *kajika*, *megalorchis*, *parasiluri*, *simile*, *sinense* and *staffordi* from which it has already been distinguished.

Phyllodistomum notropidus n. sp.

(Fig. 2)

Description: *Phyllodistomum*. Body small, spatulate, consisting of a rounded, discoidal posterior portion somewhat set off at middle of acetabulum from a narrower, tapering anterior portion. Cuticula spineless, bearing sensory papillae on suckers and on surfaces and margins of body. Posterior end of body slightly notched. Oral sucker subterminal, smaller than acetabulum; diameter of oral sucker to acetabulum as 1:1.37. Mouth subterminal, ventral; esophagus weakly muscular, slender, narrow; intestinal crura inflated, extending nearly to posterior end of body. Vitellaria oval, smooth or slightly lobate, very close to acetabulum, occasionally overlapping it. Vitelline ducts joined mesially; common vitelline duct short, slightly swollen.

Ovary compact, equal to either testis or larger, slightly and irregularly lobate, usually in contact with vitellarium, amphitypic. Oviduct short, arising from dorsum of ovary, passing antero-mesially to oötype. Mehlis' gland weakly developed, cells small. Laurer's canal arising from oviduct just before oötype and passing antero-laterally to opening on dorsal surface. Uterus fairly extensive, coils intra- and extracecal, extending to margins of body posterior to acetabulum; uterus ascending dorsal to acetabulum, constricting to weakly muscular metraterm; latter ventral to seminal vesicle; genital atrium small. Eggs fairly numerous; older intrauterine eggs containing fully developed miracidia; hatching occurring as soon as eggs enter water.

Testes oblique, fairly close together, irregularly lobate, indentations deeper and lobes more numerous than in ovary. Anterior testis directly behind but not in contact with vitellarium, opposite or very slightly oblique to ovary. Posterior testis directly behind but never in contact with ovary. Vasa efferentia arising from dorsum of testes, passing forward dorsal to acetabulum to their junction slightly anterior to acetabulum; vas deferens short; seminal vesicle large; ejaculatory duct short. Genital pore mid-ventral, posterior to intestinal bifurcation. Excretory pore postero-dorsal; bladder a slender, median tube extending anteriorly to level of ovary where right and left primary collecting ducts enter; latter extending anteriorly to intestinal bifurcation, then looping posteriorly a short distance each receiving two secondary collecting ducts.

Average measurements in millimeters (with maximum lengths and widths) of 8 specimens fixed in Bouin's fluid, stained in Mayer's paracarmine or Delafield's haematoxylin and mounted in clarite X: Body, length 1.351 (0.998–1.875), width at intestinal bifurcation 0.232 (0.195–0.285), at anterior testis 0.488 (0.405–0.645); oral sucker, diameter 0.177 (0.155–0.210); acetabulum, diameter 0.243 (0.210–0.308); esophagus, length 0.137 (0.109–0.176); left vitellarium, length 0.094 (0.056–0.133), width 0.099 (0.055–0.120); right vitellarium, 0.096 (0.067–0.133) \times 0.097 (0.069–0.112); ovary, 0.141 (0.104–0.180) \times 0.153 (0.117–0.210); older embryonated intrauterine eggs, 0.041 (0.038–0.043) \times 0.026 (0.024–0.028); anterior testis, 0.126 (0.069–0.184) \times 0.104 (0.070–0.128); posterior testis, 0.143 (0.117–0.198) \times 0.112 (0.080–0.165).

Host: *Notropis cornutus chrysocephalus* (Rafinesque).

Habitats: Ureters and urinary bladder.

Locality: Honey Creek near Ann Arbor, Washtenaw County, Michigan.

Types: U. S. Nat. Mus. Helm. Coll. No. 44949 (3 slides, type and 2 paratypes); other paratypes in author's collection.

Of 136 Mississippi Valley common shiners, 9 (approximately 6.6 per cent) were infected with mature *P. notropidis*. As many as 17 adult worms have been recovered from the ureters and urinary bladder of a single host.

Of 13 *P. notropidis* examined, 8 had the ovary on the left and 5 had it on the right, indicating that amphitopy is of frequent occurrence in this species.

Comparison with other species of the genus: An ovary equal to either testis or larger decidedly distinguishes *notropidis* from *acceptum*, *almorii*, *americanum*, *angulatum*, *carolini*, *caudatum*, *conostomum*, *elongatum*, *entercolpium*, *fausti*, *folium*, *hunteri*, *kajika*, *lacustri*, *lesteri*, *linguale*, *lohrenzi*, *lysteri*, *macrocotyle*, *marinum*, *megalorchis*, *parasiluri*, *patellare*, *pseudofolium*, *semotili*, *shandrai*, *simile*, *sinense*, *singulare*, *spatula*, *spatulaeforme*, *staffordi* and *superbum*.

It differs from *brevicicum*, *mogurndae*, *pearsei* and *unicum*, and further from *caudatum* in that the oral sucker is smaller than the acetabulum. It differs from *solidum*, and further from *lesteri*, *marinum*, *shandrai*, *spatula* and *unicum* in having an irregularly lobate ovary.

The extension of extrauterine coils as far forward as the acetabulum and laterally to the extreme margins distinguishes it from *macrobrachicola*, and also from *almorii*, *americanum*, *angulatum*, *caudatum*, *conostomum*, *elongatum*, *folium*, *lacustri*, *lesteri*, *linguale*, *lysteri*, *marinum*, *pseudofolium*, *spatula*, *spatulaeforme*, *staffordi* and *unicum*.

It differs further from *macrobrachicola* in that the body is somewhat smaller, the ovary slightly larger and the eggs significantly smaller. It has a body and ovary significantly smaller, but its eggs are significantly larger than in *undulans*.

The presence of a slight posterior notch further differentiates it from *acceptum*, *almorii*, *brevicicum*, *carolini*, *caudatum*, *conostomum*, *entercolpium*, *fausti*, *hunteri*, *kajika*, *lacustri*, *lesteri*, *linguale*, *lohrenzi*, *parasiluri*, *patellare*, *pearsei*, *shandrai*, *sinense*, *singulare*, *solidum*, *staf-fordi*, *undulans* and *unicum*. Its host, a fish, distinguishes it further from *almorii*, *americanum*, *entercolpium*, *kajika*, *lesteri*, *patellare*, *shandrai*, *singulare* and *solidum*.

P. notropidus most closely resembles *macrobrachicola*, *mogurndae*, *pearsei*, *semotili* and *solidum* from which it has already been distinguished.

Phyllodistomum nocomis n. sp.
(Fig. 3)

Description: Phyllodistomum. Body small, lanceolate, not sharply set off into narrow anterior and discoidal posterior portions. Cuticula spineless, bearing sensory papillae on suckers and on surfaces and margins of body. Posterior end of body usually slightly notched. Oral sucker subterminal, smaller than acetabulum; diameter of oral sucker to acetabulum as 1:1.39. Mouth subterminal, ventral; esophagus weakly muscular, slender, narrow; intestinal crura inflated, extending nearly to posterior end of body. Vitellaria oval, smooth, near and postero-lateral to acetabulum, occasionally overlapping it. Vitelline ducts joined mesially; common vitelline duct short, slightly swollen.

Ovary compact, very small, slightly and irregularly lobate, usually in contact with vitellarium, amphitypic. Oviduct short, arising from dorsum of ovary, passing antero-mesially to oötype. Mehlis' gland weakly developed, cells small. Laurer's canal arising from oviduct just before oötype and passing antero-laterally to opening on dorsal surface. Uterus sparse, coils mostly intracecal, extracecal coils few, sometimes extending to ovary and to margins of body; uterus ascending dorsal to acetabulum, constricting to weakly muscular metraterm; latter ventral to seminal vesicle; genital atrium small. Eggs few; older intrauterine eggs containing fully developed miracidia; hatching occurring as soon as eggs enter water.

Testes oblique, fairly close together, irregularly lobate, indentations deeper and lobes more numerous than in ovary. Anterior testis directly behind but not in contact with vitellarium; opposite or slightly oblique to ovary. Posterior testis directly behind but never in contact with ovary. Vasa efferentia arising from dorsum of testes, passing forward dorsal to acetabulum to their junction slightly anterior to acetabulum; vas deferens short; seminal vesicle very large; ejaculatory duct short. Genital pore mid-ventral, posterior to intestinal bifurcation. Excretory pore postero-dorsal; bladder a slender, median tube extending anteriorly to level of ovary where right and left primary collecting ducts enter; latter extending anteriorly to intestinal bifurcation, then looping posteriorly a short distance each receiving two secondary collecting ducts.

Average measurements in millimeters (with maximum lengths and widths) of 10 specimens fixed in Bouin's fluid, stained in Ehrlich's acid haematoxylin and mounted in clarite X: Body, length 1.016 (0.965–1.058), width at intestinal bifurcation 0.199 (0.180–0.218), at anterior testis 0.330 (0.308–0.345); oral sucker, diameter 0.140 (0.135–0.143); acetabulum, diameter 0.195 (0.180–0.205); esophagus, length 0.118 (0.093–0.135); left vitellarium, length 0.046 (0.037–0.061), width 0.069 (0.045–0.077); right vitellarium, 0.050 (0.040–0.064) \times 0.073 (0.053–0.093); ovary, 0.066 (0.059–0.080) \times 0.076 (0.059–0.096); older embryonated intrauterine eggs, 0.040

(0.037–0.045) \times 0.026 (0.024–0.027); anterior testis, 0.103 (0.093–0.120) \times 0.087 (0.053–0.109); posterior testis, 0.129 (0.120–0.139) \times 0.096 (0.061–0.113).

Host: *Nocomis biguttatus* (Kirtland).

Habitats: Ureters and urinary bladder.

Locality: Honey Creek near Ann Arbor, Washtenaw County, Michigan.

Types: U. S. Nat. Mus. Helm. Coll. No. 44948 (3 slides, type and 2 paratypes); other paratypes in author's collection.

Of 2 horny-headed chubs, 1 was infected with 31 mature *P. nocomis*. The adult worms were recovered from the ureters and urinary bladder.

Of 28 *P. nocomis* examined, 10 had the ovary on the left and 18 had it on the right, indicating that amphitropy is of frequent occurrence in this species.

Comparison with other species of the genus: A significantly smaller ovary distinguishes *nocomis* from all other species of *Phyllodistomum*. Smooth vitellaria differentiate it further from *acceptum*, *americanum*, *angulatum*, *brevicicum*, *carolini*, *caudatum*, *conostomum*, *elongatum*, *entercolpium*, *fausti*, *folium*, *hunteri*, *lacustri*, *linguale*, *lysteri*, *mogurndae*, *parasiluri*, *patellare*, *pearsei*, *pseudofolium*, *sinense*, *singulare*, *spatula*, *spatulaeforme*, *staffordi*, *superbum* and *undulans*.

A slightly and irregularly lobate ovary differentiates it further from *lesteri*, *marinum*, *shandrai*, *solidum*, *spatula* and *unicum*. It differs from *brevicicum*, *caudatum*, *mogurndae*, *pearsei* and *unicum* in that the oral sucker is smaller than the acetabulum. With the exception of *angulatum*, *elongatum*, *lacustri*, *lesteri*, *linguale*, *macrobrachicola* and *staffordi* a sparsely coiled uterus distinguishes it from all other species, including *almorii*, *kajika*, *lohrenzi*, *megalorchis*, *notropidus*, *semotili* and *simile*.

A significantly smaller body and eggs, and vitellaria that are only slightly smaller than the ovary differentiate it further from *macrobrachicola*. It differs further from *macrocotyle* in having a significantly smaller body, testes and eggs. Its host, a fish, distinguishes it further from *almorii*, *americanum*, *entercolpium*, *kajika*, *lesteri*, *patellare*, *shandrai*, *singulare* and *solidum*.

P. nocomis most closely resembles *almorii*, *angulatum*, *elongatum*, *kajika*, *lesteri*, *linguale*, *macrobrachicola*, *notropidus* and *semotili* from which it has already been distinguished.

BIBLIOGRAPHY

- GROAT, R. A. 1939 Two new mounting media superior to Canada balsam and gum damar. *Anat. Rec.* 74: 1–6.
- HOLL, FRED J. 1929 The phyllodistomes of North America. *Tr. Am. Micr. Soc.* 48: 48–53.
- LEWIS, F. J. 1935 The trematode genus *Phyllodistomum* Braun. *Tr. Am. Micr. Soc.* 54: 103–117.
- NYBELIN, O. 1926 Zur Helminthenfauna der Süßwasserfische Schwedens I. Phyllodistomen. Göteborgs K. Vetensk.-o. Vitterhets-Samh. Handl. 4th Ser. 31: 1–29.
- WU, KUANG 1938 Progenesis of *Phyllodistomum lesteri* sp. nov. (Trematoda: Gorgoderidae) in freshwater shrimps. *Parasitology* 30: 4–19.

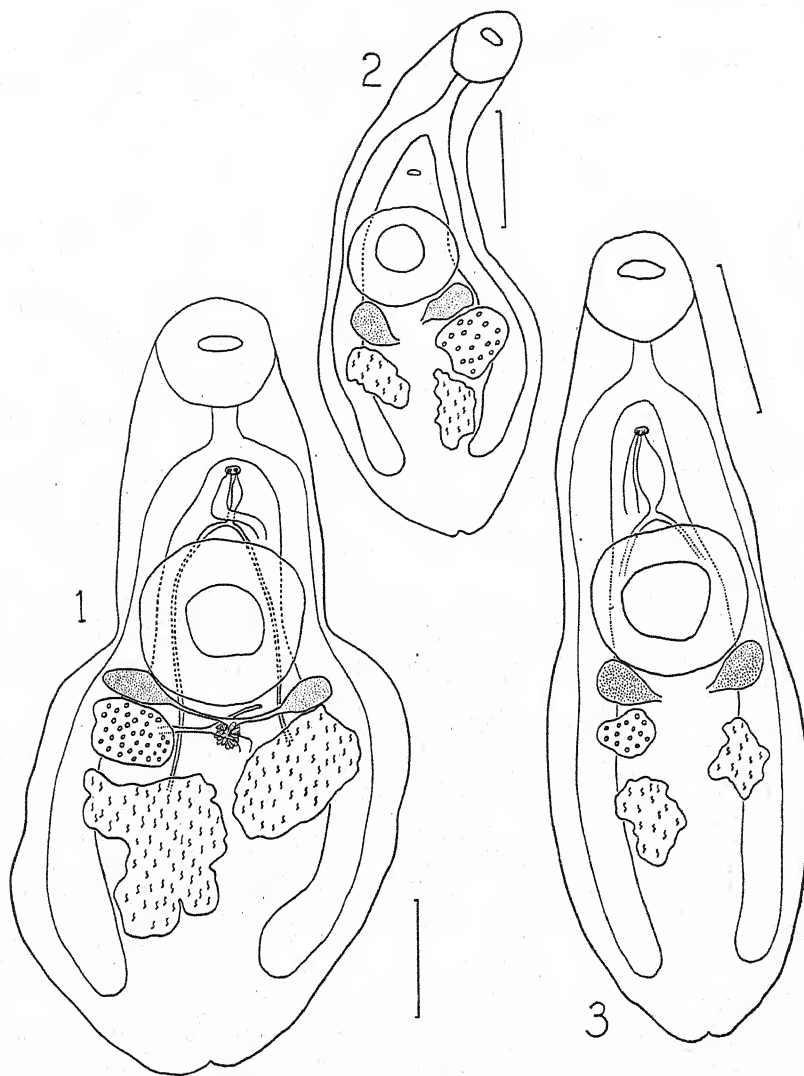
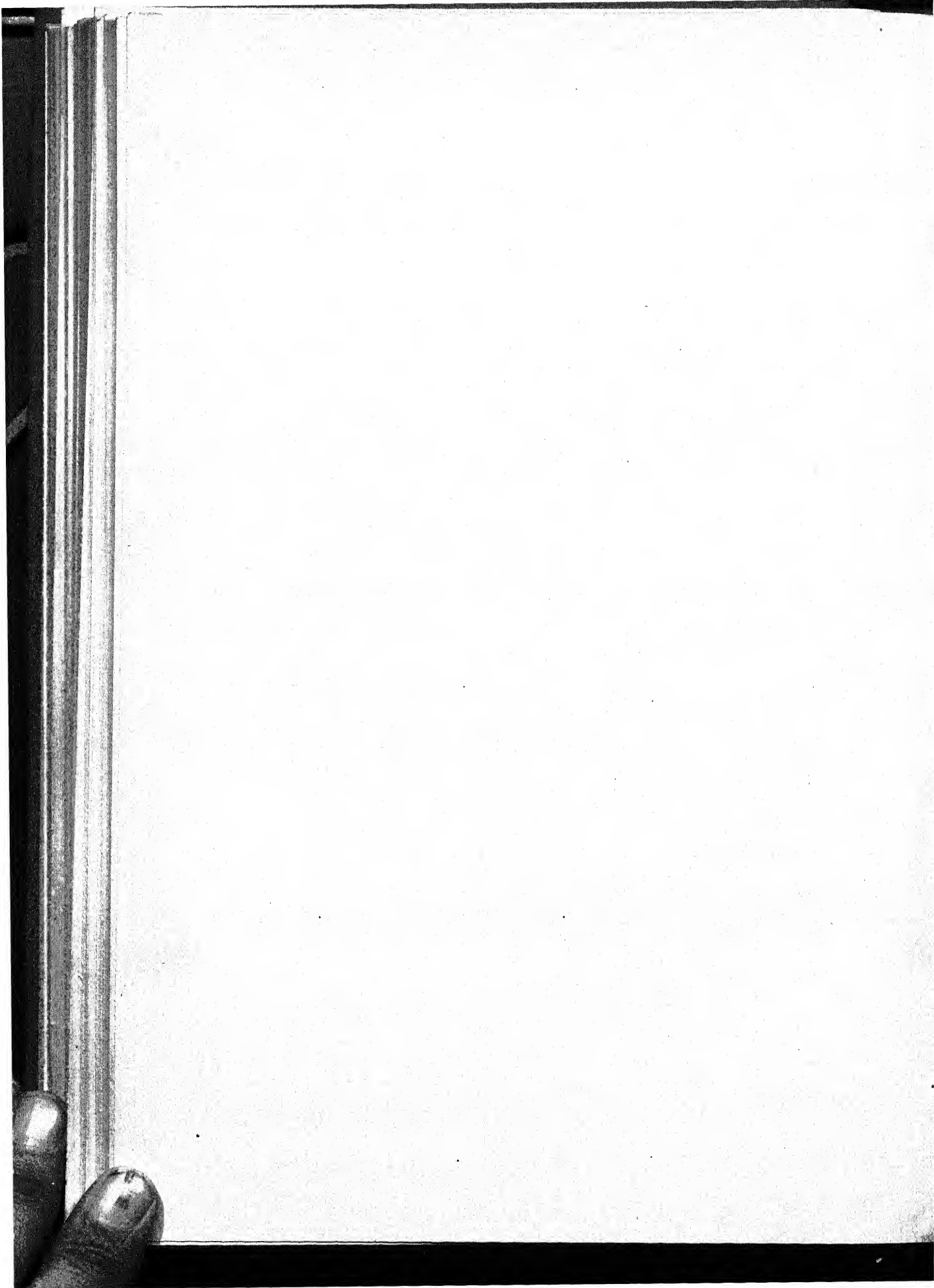


PLATE I

All figures drawn to scale with the aid of a microprojector; value of scale is 0.2 mm.

- FIG. 1. *Phyllodistomum semotili*, adult, ventral view.
FIG. 2. *Phyllodistomum notropidis*, adult, ventral view.
FIG. 3. *Phyllodistomum noconis*, adult, ventral view.



THE LIFE HISTORY OF *POROCEPHALUS CROTALI*, A PARASITE OF THE LOUISIANA MUSKRAT¹

GEORGE H. PENN, JR.

Wildlife and Fisheries Division, Louisiana Department of Conservation

A preliminary report of the incidence of nymphal porocephaliasis of the Louisiana muskrat has already been published (Penn and Martin, 1941). The present paper is to report what is known of the life history of *Porocephalus crotali* in Louisiana as a result of studies in the past year, and to add a few additional data on the incidence and epidemiology.²

HOSTS AND DISTRIBUTION

Porocephalus crotali was first described by Humboldt in 1808 as *Echinorhynchus crotali*. Other important synonyms for the adult are *Pentastoma proboscideum* Rudolphi, *Porocephalus humboldtii* Mayer, *Linguatula quadriuncinata* Mayer and *Pentastoma moniliforme* Megnin; and, for the nymphal form, *Pentastoma subcylindricum* Diesing.

Porocephalus crotali, according to Sambon (1922), has a limited distribution, the adults being found principally in New World crotaline snakes, especially the species of *Crotalus* which "range from Arizona, New Mexico and Texas to southern Brazil and northern Argentina." The type specimen was taken from the South American rattlesnake, *Crotalus durissus terrificus* Laurenti. It has also been recorded from *C. durissus durissus*, *C. atrox*, *C. horridus* ssp., and *C. adamanteus*. Dr. Howard R. Hill at the Los Angeles Museum states³ that he has found *P. crotali* commonly in the water-moccasin (*Agkistrodon piscivorus*); this reptile has also been recorded as a host of the adult by the writer (1940).

Sambon (1922) and Heymons (1935) state that the nymphal form has been reported from several Brazilian mammals (MARSUPIALIA: the

Received for publication, September 8, 1941.

¹ A contribution from the Department of Zoology, the Tulane University of Louisiana, New Orleans.

² It is a pleasure to acknowledge the cooperation of Dr. Carl E. Venard, Ohio State University, for identification of the pentastomes; of Florence Brooks Kreeger and Dr. Harley N. Gould, Tulane University, in securing some of the muskrats; of Harry E. Adams, Julian A. Howard and the rest of the personnel of the Sabine National Wildlife Refuge, Louisiana, in supplying muskrats; and of Ruffin T. Perkins, Jr., in securing live water-moccasins and other experimental animals. Dr. F. H. Wilson, Tulane University, read and criticized the manuscript and offered a number of valuable suggestions.

³ In a personal communication, September 24, 1940.

murine opossum *Marmosa murina* L. and *Caluromys philander* L.; CHIROPTERA: *Phyllostoma discolor* Wagn.; EDENTATA: *Tatusia novemcincta* L.; CARNIVORA: *Procyon cancrivorus* Cuv. and *Pteronura brasiliensis* Zim.; SIMIAE: *Mystax chrysopygus* Wagn.; and RODENTIA: *Rhipidomys pyrrhorhinus* Wied and *Akodon fuliginosus* Wagn.), but that the determination of the parasites has been uncertain in the majority of the cases. The nymph was first recorded from the viscera of the Louisiana muskrat (*Ondatra zibethica rivalica*) by the writer (1940).

THE LIFE HISTORY

Adults live in the lung cavities of the reptilian host. The eggs, which reach the outside in sputum, are ingested by the muskrat and hatch in the small intestine. The larvae migrate to the liver and lungs of the muskrat where they become encapsulated in the tissues, and develop into nymphs in about three months. The tissue with infective nymphs is then ingested by the reptile host and the nymphs migrate up the esophagus and into the tracheae and lungs where they develop into adults.

THE EGGS

Description.—The eggs, Fig. 1, are fully developed when deposited by the female porocephalid inside the pleural cavity of the snake host. The fully formed larva is enclosed in an inner oval-shaped vitelline membrane which in 12 eggs averaged 123 microns long (from 114 to 128) and 105 microns wide (99 to 106). The outer membranous shell is much larger, perfectly spherical in shape, and has a wide clear area between it and the inner membrane; its diameter averaged 151 microns in 12 eggs (from 135 to 170).

Productivity.—The number of eggs contained in the uterus of a 64 mm female porocephalus was calculated to be approximately 1,088,000. This must be considered as only a rough estimate as it was made by measuring the length of the uterus containing eggs and cutting out pieces as nearly as possible one millimeter long. The sections were then measured exactly and the eggs squeezed out on slides and counted exactly. Because the uterus is the same diameter throughout its length, and the eggs are nearly the same size regardless of the stage of development, the average of only four such counts (which showed little variation) was used in the final calculation. The uterus of this female was 918 mm long and the count per 0.1 mm averaged 118 eggs (100 to 150).

Viability.—On August 18, 1940 a gravid female *P. crotali* just removed from a water-moccasin was cut into shreds and placed in about eight ounces of marsh-lagoon water diluted with well-water to a salinity of approximately 0.006% (equal to 0.32% sea water). From time to time as the worm decayed the suspension of eggs was examined micro-

scopically and all of the embryonated eggs found viable. The last so examined, 189 days after having been removed from the living parent, hatched into motile larvae on slides under observation.

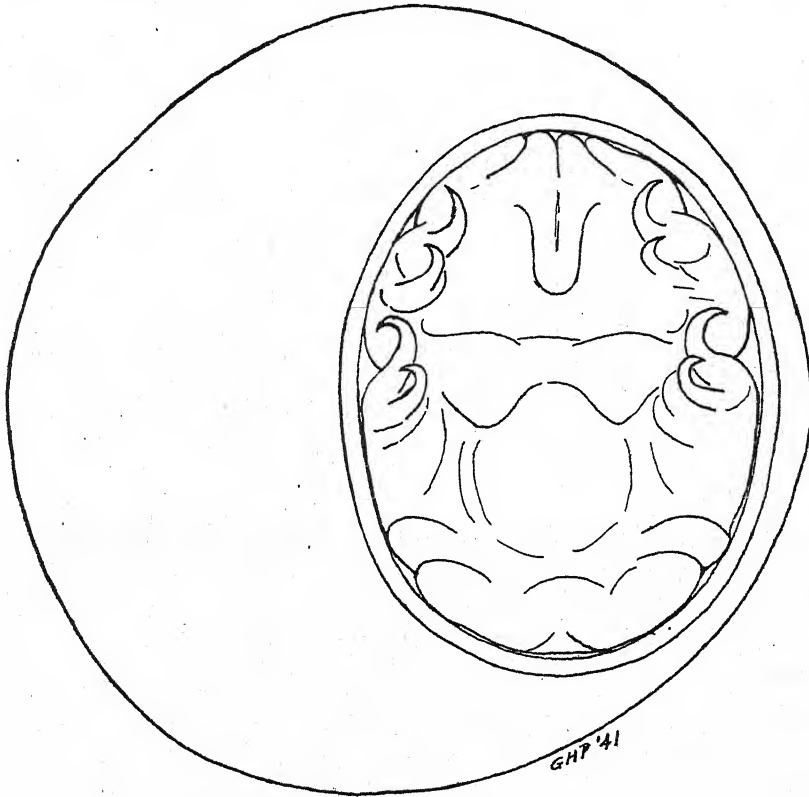


FIG. 1. *Porocephalus crotali*: embryonated egg.

THE LARVAE

Hatching.—The eggs hatch by two stages (as observed when they were placed on slides under cover glasses). First, the inner vitelline membrane splits into halves from the head end, and the larva, Fig. 2, stretches to its full size of approximately 141 microns long and 64 microns wide, while still inside the outer shell. After an indefinite period of more or less constant action by the larva, the outer shell is split into halves in much the same fashion as was the inner shell, and the larva emerges. By continued slow, strong strokes with its four, doubly hooked legs the larva frees itself of the egg cases and progresses on its migration to the site of encapsulation.

Migration of the Larvae.—From several experiments in which laboratory white rats were killed at a series of short intervals after being

orally infected with porocephalid eggs, the place of hatching was determined to be the duodenum. Both embryonated and non-embryonated eggs were used so that the level reached by the eggs was always marked by the presence of non-embryonated eggs at least. One hour after feeding, both types of eggs were recovered from the stomach, but none in the intestine. After two and four hours respectively non-embryonated eggs were recovered in the duodenum and jejunum but none of the others. Evidently all embryonated eggs hatched in the duodenum.

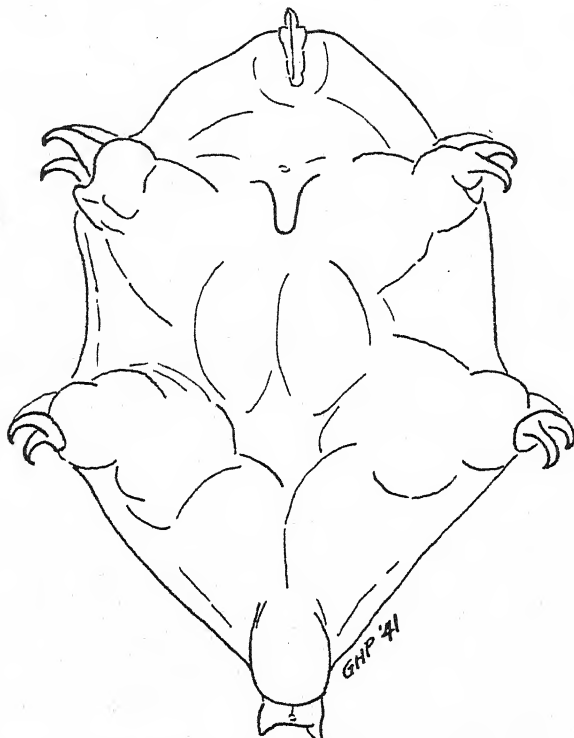


FIG. 2. *Porocephalus crotali*: larva, just hatched; ventral side.

No larvae were recovered from any of the rats, although each examination included the lumen of the stomach, duodenum, jejunum, the peritoneum of the stomach, duodenum, jejunum and surfaces of the liver, therefore leaving the route of migration still a matter of conjecture. Because of the size of the larvae they cannot pass through the blood capillaries of the hepatic-portal system, and a blood route therefore seems out of the question. The most probable route seems to be either through the bile ducts from the duodenum to the hepatic tissue and from there to the surface of the liver, or through the intestinal wall directly into the abdominal cavity and then to the surfaces of the liver and other viscera.

The latter, although unsupported by experimental data, appears the more favorable to the writer especially when the relatively larger number of nymphs which encapsulate in the omenta of experimental white rats is considered. A direct route through the wall of the duodenum to the omenta seems indicated.

THE NYMPHS

When the larva reaches the end of its migration, which is usually in the liver, it continues its post-larval development. It becomes imbedded in the tissue in which it has settled and shortly afterward is encapsulated by host-tissue reaction. Within the capsule the larva develops into a worm-like nymph. The nymph grows and enlarges the capsule until it is fully developed in about 100 days.

Experimental.—White rats forced to ingest viable eggs on August 27th were heavily infected with nearly developed nymphs 93 days later on November 29th. These nymphs were smaller than those obtained from muskrats, and were probably not quite fully developed; nevertheless, some of these lived in the experimental reptile host, two out of three baby water-moccasins being successfully infected with 15 mm immature porocephalids after 35 days. On the other hand, all of the encapsulated nymphs (of unknown ages) obtained from muskrats on the same date were infective and developed into post-nymphal forms when fed to water moccasins.

The fully developed nymph makes a capsule of 5.0 mm (from 4.5 to 6.0) diameter, usually in the liver of the muskrat, but also possibly in or on every organ of the visceral and pleural cavities. Only one or two nymphs are normally present in a naturally infected muskrat, where they are almost invariably located at the periphery of the hepatic tissue; the lungs, omentum and gonads, however, also are frequently parasitized, and in that order of incidence.

The nymphs themselves when removed from the capsules averaged 12.2 mm long (from 11.0 to 13.0) in the specimens measured. The number of annulations varied between 38 and 42, averaging about 40, on twenty nymphs obtained from naturally infected muskrats.

The possibility that additional hosts of the nymphal form exist in Louisiana has not been thoroughly investigated as yet, although three *Rattus norvegicus* and nine *Mus musculus* from the vicinity of the Sabine Refuge headquarters examined by the writer were found negative. Other small marsh rodents of the same locality also should be examined for the nymphal form of this porocephalus.

THE ADULTS

In laboratory-raised, baby water-moccasins four months old and in wild water-moccasins collected at Westwego, La., not over six months old

(26.0 to 27.0 cm long) it was possible to culture post-nymphal or immature-adult *Porocephalus crotali*.

Among these experimental water-moccasins four were forcibly fed with encapsulated nymphs obtained fresh from muskrats trapped at Abbeville, La., November 29th. All of the snakes proved to be satisfactory hosts for the development of the post-nymphal stages. However, one of the snakes was found dead on January 3rd with two 20 mm immature porocephalids clogged in its trachea, suffocation probably being the cause of death. Three water-moccasins of the same lot were infected orally with encapsulated nymphs from experimental white rats as described in the preceding section.

Twenty-five water-moccasins (*Agkistrodon piscivorus*) collected near the headquarters of the Sabine Refuge in Cameron Parish, La., were autopsied between August 12th and 19th, 1940. Seven were infected with adult and immature porocephalids, a higher percentage of females than males being infected. A few pentastomes of the species *Kiricephalus coarctatus* also were obtained from these moccasins. The range of the moccasins was from 39 to 120 cm, 17 were males, 7 females and one juvenile. Three additional water-moccasins from the Chalmette and Pearl River swamps in eastern Louisiana proved to be negative for *Porocephalus*. Twelve alligators ranging from 18 inches to five feet in length and a wild hog from the Sabine Refuge also were examined for adult porocephaliasis, but found negative.

In the laboratory various cold-blooded vertebrates were forcibly infected per os with encapsulated nymphs freshly obtained from wild muskrats. The experimental animals were examined twelve days after infection. Of three water snakes (*Natrix sipedon confluens*) two became infected; of three ribbon snakes (*Thamnophis sauritus proximus*) two became infected; one mobilian turtle (*Pseudemys scripta troosti*) became infected; of two small green frogs (*Rana clamitans*) one became infected. One mud-turtle (*Sternotherus odoratus*), one baby alligator (*Alligator mississippiensis*) and one tree frog (*Hyla cinerea cinerea*) failed to become infected.

OCCURRENCE OF THE NYMPHS IN MUSKRATS

Preliminary notes of porocephaliasis in Louisiana muskrats by Penn and Martin (1941) gave the figure of 9.06% as the incidence for the state of Louisiana in the 1939-40 trapping season. Of an additional 748 muskrats examined in the 1940-41 trapping season, 11.5% were found with porocephaliasis.

Data of the sexes, size and degree of infection of the muskrats were collected in the latter survey. Only a slight difference between sexes is shown, the females having a slightly higher percentage of infection than

the males. It is interesting to note also that only the larger, i.e., sexually mature (25 to 33 cm) muskrats were infected although many smaller ones were examined from the same areas.

Geographic difference in intensity of infection in 1940-41 similar to that reported in the previous study again indicates that porocephaliasis is dependent on the relative numbers of hosts of the parasite living in any particular marsh. It is obvious that the heavier the concentration of muskrats or of water-moccasins, or both on any area, the greater will be the chance for infection to increase in both hosts. Concentrations may be caused by natural fecundity of the muskrats coupled with abundance of native food-grasses and water, or may occur in drought years when muskrats congregate in the vicinity of small pools of fresh water. In either instance, the water-moccasins will be found seeking their prey and at the same time distributing the eggs of the parasite. In regard to moccasins in marshes, Arthur (1931) wrote that it "has been an old adage in the Louisiana marshes that wherever the poisonous moccasin snake is plentiful one is certain to find many muskrats. On the other hand, some experienced and practical trappers will tell you that the moccasins become so plentiful and take such toll of young that the muskrat population is kept down."

BIBLIOGRAPHY

- ARTHUR, STANLEY C. 1928 The fur animals of Louisiana. Louisiana Dept. Cons., Bull. No. 18.
- GLOYD, HOWARD K. 1940 The rattlesnakes, genera *Sistrurus* and *Crotalus*. Chicago Acad. Sc., Spec. Pub. No. 4: 1-266.
- HEYMONS, R. 1935 Pentastomida. In Bronn's Klassen und Ordnungen des Tierreiches, 5, Abt. 4, Lief 1-2: 229-233.
- PENN, GEORGE H., JR. 1940 Observations on the life cycle of a linguatulid, *Porocephalus crotali* (Humboldt 1808). Anat. Rec. 78 Suppl.: 125.
- PENN, GEORGE H., JR. AND MARTIN, ERNEST C. 1941 The occurrence of porocephaliasis in the Louisiana muskrat. J. Wildlife Management 5: 13-14.
- SAMBON, L. 1922 A synopsis of the family Linguatulidae. J. Trop. Med. 25: 188-206; 391-428.

THE METACERCARIA AND ADULT OF *CENTROCESTUS FORMOSANUS* (NISHIGORI, 1924), WITH NOTES ON THE NATURAL INFECTION OF RATS AND CATS WITH *C. ARMATUS* (TANABE, 1922)

H. T. CHEN¹

Division of Biology, Lingnan University, Canton²

In the fall of 1939 when an extensive examination of local fish was made for certain trematode infections a large number of oval metacercariae characterized by double circles of circumoral spines and a very distinct X-shaped excretory bladder was found in the gills of *Macropodus opercularis* and *Puntius semifasciolatus* which were collected from ponds and pools of Tsuen Wan and Fanling, villages between Kowloon and the Chinese-British border in the New Territories of Hong Kong. Feeding of these cysts to white rats resulted in the recovery a week later of many adult trematodes of the species *Centrocestus formosanus* (Nishigori, 1924), a heterophyid reared experimentally in man in Formosa and a potential human parasite in this region.

Mr. C. T. Cheng of this division called the author's attention to similar cysts in the stomach wall and striated muscles of the frog, *Rana limnocharis*, from Kowloon. These likewise proved to be *C. formosanus* when reared in white rats.

HISTORICAL ACCOUNT

Centrocestus was first created by Looss (1899) to include *C. cuspidatus* described by him in 1896 under the name *Distomum cuspidatum*. In 1913 Leiper described as *C. cuspidatus caninus* a fluke from Formosa which is morphologically the same as *C. cuspidatus* but with only 28 spines surrounding the mouth. This name was considered a synonym of *C. cuspidatus* by Ransom (1921). Tanabe (1922) erected a new genus, *Stamnosoma*, for the reception of a new fluke from Japan, *Stamnosoma armatum*, which is closely similar to *Centrocestus*. Following the same generic concept Nishigori (1924) described *S. formosanum* from northern Formosa.

Chapin (1926), Fuhrmann (1928), Witenberg (1929), Price (1932), and Stiles and Baker (1934) all consider *Stamnosoma* to be a synonym

Received for publication, September 22, 1941.

¹ Thanks are due to the following friends for securing certain literature not available in Hong Kong: Dr. C. M. Africa, Dr. H. F. Hsu and Dr. S. M. K. Hu. Mr. S. Y. Lin of the Hong Kong Fisheries Research Station has kindly identified the fish hosts for the writer. To Dr. F. G. Wallace I am in appreciation for assistance in preparing the manuscript.

² The University was temporarily located in Hong Kong after November, 1938.

of *Centrocestus*. Faust and Nishigori (1926) accepted the genus *Stamnosoma* and considered that Leiper's specimen of *C. cuspidatus caninus* was really *S. formosanum*. Later, however, Faust (1937) discarded the name *Stamnosoma* in favor of *Centrocestus*.

The metacercarial stages of *C. armatus* and *C. formosanus* have been briefly described by Tanabe (1922) and Nishigori (1924) respectively. Yamaguti (1938) added to our knowledge of the metacercariae and earlier stages of *C. armatus*. *C. formosanus* has been reported from the Philippines by Vazquez-Colet and Africa (1939, 1940) and Africa, de Leon and Garcia (1940). In addition Vazquez-Colet and Africa (1938) report from a marine fish in the Philippines, *Ambassis buruensis* (lañgaray), two apparently different species of *Centrocestus* (one big and having a long intestine, and the other very tiny and having a short intestine). The larger of the two also parasitizes another marine fish, *Hepsetia balabacensis*. The authors consistently failed to infect albino rats and mice, kittens and puppies, indicating apparently that the two metacercariae are distinct forms.

Because of the brevity of extant descriptions the metacercaria of *Centrocestus formosanus* is fully described here and certain obscure morphological aspects of the adult are elucidated.

In China the genus was first found in *Mus norvegicus* in 1933 by the writer and was reported from domestic cats (Chen, 1934), both from Canton.

THE METACERCARIA

The following descriptions are based primarily on material from fish unless otherwise stated, although there are no appreciable differences between these and specimens from frogs.

The cysts are most frequently found attached to the gill filaments, occasionally in the operculum and very rarely in the muscle or on the scales, showing that they prefer tissues in which there is an abundant supply of blood and oxygen. For instance, in a series of 12 *M. opercularis* examined for cysts, one each was found on the scales of 3 fishes, from 1 to 4 on the operculum of 4 fishes, 3 in the muscle of one host, and from 2 to 527 (averaging 126 per fish) on the gills. In frogs they are primarily located in the stomach wall, but may often be found in the muscles.

The cyst is oval and consists of a single-layered, strong, transparent wall about 2μ thick (Fig. 7). Fifty-two living cysts in water without pressure measured from 0.132 to 0.204 mm (av. 0.176 mm) long and 0.105 to 0.168 mm (av. 0.127 mm) wide. The cyst is always covered by a thick layer of the reaction tissue of the host. With the latter intact 42 cysts gave while alive an average of 0.208 by 0.154 mm.

Excystation can take place easily when the cyst is placed in pepsin digestive fluid for about 2 hours at 37° C and is then placed in pancreatic juice. The thin wall becomes hyaline and swollen to a thickness of about 5 μ , and then is broken by a constant stretching of the parasite. Excysted individuals (Fig. 8) are very active, and it is difficult to measure the body. Under slight pressure the measurements of 8 individuals were 0.267–0.386 mm (av. 0.301 mm) long and 0.099–0.200 mm (av. 0.140 mm) wide. The entire body is studded with short but conspicuous spines. The oral sucker is 0.053 mm wide by 0.058 mm long. The acetabulum is located at about the middle or posterior to the middle of the body and measures 0.033–0.049 mm (av. 0.035 mm) wide and 0.025–0.040 mm (av. 0.031 mm) long. These figures were obtained from 9 specimens.

One of the most characteristic features of this species is the two circles of circumoral spines (Figs. 8, 9), each somewhat recurved at the tip. Those of the outer circle are larger and measure 0.012–0.015 mm long while those of the inner circle are slightly shorter, generally from 0.010–0.012 mm, the width at the base being about 0.002 mm in both. It appears also that those on the ventral side are slightly smaller than the dorsal and are closer together but this point requires more extensive comparisons. The number of spines varies from 30 to 36 with 31 and 32 the commonest. The spines fall away fairly easily and care must be exercised in evaluating their total number as a specific character.

The mouth is large and opens into a funnel-like cavity. The prepharynx (Fig. 8) is practically absent in a very active specimen, but as it becomes sluggish the prepharynx gradually makes its appearance. The average length of the prepharynx of such specimens measured under some pressure of a cover glass is 0.021 mm. The pharynx in 11 specimens measured 0.029 mm wide and 0.033 mm long. Of those which are examined with considerable pressure the pharynx may become wider than long. It is divisible into two parts. The anterior which constitutes the bulk of the structure is muscular, while the posterior third or fourth is granular and is glandular in function. In vitally stained specimens the posterior part is deeply colored while the anterior is colorless (Fig. 12). The esophagus is practically absent in unextended specimens. The average length of 5 was 0.014 mm. The two ceca end either at the posterior border of the acetabulum or between the latter and the anterior border of the excretory bladder. The intestinal ceca may be filled with crystals and are sometimes enlarged posteriorly.

The nervous system consists of a pair of ganglia located on each side of the pharynx or prepharynx and connected by a transverse commissure (Fig. 8). Nerve fibers go upward from each of the ganglia and disappear from view before reaching the oral sucker, while others go downward, cross the ceca in front of the acetabulum, and disappear before reaching the level of the testes.

The reproductive system (Fig. 8) consists of well-developed organs which require only a day or so to become fully developed in the definitive host. The male system consists of two approximately oval testes, slightly lobed at the outer or posterior border, generally placed somewhat obliquely or transversely opposite each other at the posterior end of the body. The left testis measured 0.066 by 0.046 mm and the right 0.065 by 0.460 mm in 10 specimens. Two small ducts lead forward and join together to form the seminal vesicle which is large and consists of two parts divided by a constriction, both located more or less transversely behind the acetabulum. The proximal portion or the posterior vesicle turns from right to left, and the distal portion or the anterior vesicle turns sharply back from left to right. The latter follows the curve of the acetabulum on its lower right border to join the prostate section which is followed by the terminal portion along the anterior right border of the acetabulum. It opens into the common genital atrium in front of the acetabulum. The genital pore can be seen as a transverse slit.

The female system is not as clear as the male system. It consists of a more or less spherical or oval ovary, about half the size of the testes, on the right side just in front of the right arm of the excretory bladder. An oviduct begins from the dorsal side of the ovary, passing transversely to the middle on the ventral side of the seminal receptacle, receiving a short duct from the latter somewhere at the center and connecting with the proximal end of the uterus. Laurer's canal appears to open into the seminal receptacle.

There is a group of banana-shaped skin glands (Figs. 10, 11). Each is connected with a short duct and opens to the outside through the skin. The glands are found all over the body except in the posterior portion. On the ventral side they are arranged in five longitudinal rows. The central row consists of from two to five glands between the oral and the ventral suckers. The lateral rows, each consisting of about 15 glands, extend from below the oral sucker to a level in front of the excretory bladder. The two marginal rows, each consisting of about half a dozen or so glands, occupy the region between the oral and ventral suckers. Variations in this arrangement have been noted.

There is also a group of cephalic glands (Fig. 8) which are crowded together in grape-like aggregations on the sides of the esophagus, the outer margin of the ceca, and between the intestinal bifurcation and the ventral sucker. Some glands are seen between the ventral sucker and the excretory bladder. All the gland cells are granular and do not take vital stains, such as neutral red, Bismarck brown, Nile blue sulphate, or methylene blue. They vary in shape because of crowding.

A complicated system of granular ducts is faintly visible but is extremely difficult to work out (Fig. 8). They are apparently derived

from gland cells at different levels and are massed together at the level of the esophagus and pharynx. At the level of the pharynx the outermost ducts become separated from the main bundle and pass antero-laterally. Immediately anterior to the pharynx all ducts diverge and pass forward dorsally, laterally, and latero-ventrally to the oral sucker to open near the circumoral spines. The ducts are not uniform in diameter but are enlarged here and there so that they appear like strings of beads. They are larger in diameter near the oral sucker.

The excretory system (Fig. 8) is characterized by a very conspicuous X-shaped excretory bladder. From each side of the bladder a convoluted collecting tubule extends upward to the level of the pharynx and then bends down with its diameter much decreased. Its further course cannot be traced. Eight pairs of flame cells were found in all specimens studied. Their arrangement is shown in Fig. 8.

Pigment granules of irregular size are found ventrally and dorsally in the middle region of the body.

FISH HOSTS

In Formosa (Nishigori, 1924): *Carassius auratus* (Linn.), *Clarias fuscus* (Lacépède), *Channa formosana* (Jordan and Evermann), *Pseudorasbora parva* (Schlegel), *Rhodeus occelatus* (Kuer), *Gambusia affinis* (Baird and Girard), *Polyacanthus operculatus* (Linn.), *Ctenopharyngodon idellus* (C. and V.), *Ophicephalus tadianus* (J. and E.), *Misgurnus anguillicaudatus* (Cantor), *Parasilurus asotus* (Linn.), *Zacco platypus* (T. and S.) and *Cyprinus carpio* (Linn.).

In the Philippines (Vazquez-Colet and Africa, 1939): *Ophicephalus striatus* Bloch, *Glossogobius giurus* Buchanan-Hamilton, *Therapos plumbeus* (Kuer) and *Anabas testudineus* (Bloch.); (Vazquez-Colet and Africa, 1940): *Hemiramphus dussumieri* Cuvier and Valenciennes.

In Hong Kong: *Carassius auratus* (Linn.) from Fanling, 63 per cent infected; *Macropodus opercularis* (Linn.) from Fanling 82 per cent and from Tsuen Wan 91 per cent infected; *Puntius semifasciolatus* (Gunther) from Fanling 70 per cent and from Tsuen Wan 93 per cent infected. *Limia caudofasciata* Regan was experimentally infected in the laboratory. The incidence of infection in *Rana limnocharis* Gravenhorst from one locality at Kowloon is less than 2 per cent and from another 25 per cent. The intensity of infection in *Puntius* is from 2 to 288 cysts, with an average of 32 per host, in *Macropodus* 2 to 527 with an average of 128 per host, in *Carassius* 2 to 31 cysts, with an average of 7 per host. In frogs the intensity of infection is from 2 to 40.

In addition *Misgurnus anguillicaudatus* (Cantor), another freshwater fish, and *Bufo melanostictus* Schneider, the common toad, are also infected; their incidence and intensity of infection not being recorded.

TABLE 1.—Results of feeding experiments with *Centrocestus* cysts from various sources

Experimental host	Source of cysts	No. of cysts	Days after feeding	Worms recovered	Maturity
<i>Mus rattus</i>	<i>P. semifasciolatus</i>	428	4	67	Eggs present
White rat	"	288	31	9	" "
"	"	1010	3	204	" "
"	<i>Rana limnocharis</i>	20	2	4	" "
Duckling	<i>P. semifasciolatus</i>	278	3	10	" "
"	<i>M. opercularis</i>	320	3	A few	" "
Chicken	<i>C. auratus</i>	38	3	4	Immature
"	<i>P. semifasciolatus</i>	86	8	3	Eggs present
White mouse	"	401	8	35	" "
"	"	125	24	31	" "
"	<i>M. opercularis</i>	102	26	4	" "
Rabbit	"	197	15	6	" "
"	<i>P. semifasciolatus</i>	430	45	13	" "
Puppy	<i>M. opercularis</i>	611	58	7	" "
Kitten	<i>Funus</i> and <i>Macropodus</i>		11-17	14	" "

THE EXPERIMENTALLY OBTAINED ADULT

Because there are as yet no available descriptions of this species in other languages than Japanese and because there are several structures which require further elucidation, the following description is given, with measurements in millimeters. The metacercaria of this species is very advanced in development, so what has been said concerning this stage is generally applicable to the adult.

The results of feeding experiments with various final hosts are presented in Table 1; sample measurements in Table 3.

Centrocestus formosanus (Nishigori, 1924)

Description: Body flask-shaped, somewhat flattened dorso-ventrally. Twenty specimens measured 0.314-0.572 (av. 0.461) long by 0.171-0.242 (av. 0.212) wide. Oral sucker terminal, 0.056 wide by 0.060 long. Acetabulum at middle of body, 0.055 wide by 0.052 long. Oral sucker armed with two circles of spines somewhat recurved near tip and directed backward, numbering generally 30-32 and occasionally 33-36 (Figs. 1, 2, and 3). Inner circle with larger spines each measuring 0.010 to 0.013 long and 0.002-0.003 broad at base; outer circle with spines 0.008-0.010

TABLE 2.—Showing the age of the trematode as related to the number of eggs present

No. of worms	Host	Age in days	No. of eggs present
3	White rat	1.5	0, 2, 3
7	"	3-7	3, 4, 4, 5, 6, 8, 16
14	"	10	2, 6, 9, 9, 10, 11, 11, 12, 13, 14, 17, 20, 25, 28
3	"	12	21, 28, 32
2	Rabbit	15	4, 4
8	Kitten	11-17	1, 11, 12, 12, 15, 18, 18, 24
9	Mouse	25	1, 2, 2, 3, 3, 5, 5, 5, 7
2	White rat	30	20, 20
6	Rabbit	45	16, 17, 18, 21, 23, 48
5	Puppy	58	10, 17, 22, 22, 25

TABLE 3.—Measurements of various parts of the two specimens from rats.
In millimeters

	Specimen 1	Specimen 2
Body	0.598 by 0.360	0.583 by 0.274
Oral sucker	0.088 wide	0.060 wide
Prepharynx	0.028 long	0.053 long
Pharynx		0.046 wide
Testes	0.123 by 0.077	0.105 by 0.060
Ovary		0.063 diameter
Seminal receptacle	0.122 by 0.086	0.098 by 0.070
Vitellaria	From level of prepharynx to posterior end of body	Same as in specimen 1
Eggs	0.035 by 0.023. Over 60 eggs altogether	0.034 by 0.024. As many as in specimen 1

long and about 0.002 broad at base. In some specimens smaller chitinous rodlets, or sometimes true spines, either singly or in pairs, may be found irregularly between spines of outer or inner circle. In specimens one month or more old, spines are larger, inner being 0.019 by 0.005 and outer 0.016 by 0.003, and tips are mostly worn off.

Mouth opens into prepharynx which is about 0.072 long by 0.032 wide. Pharynx 0.42 wide by 0.041 long, and followed by very short esophagus. Ceca large and extend to level of middle of ovary.

Two testes, oval, slightly lobed, on posterior border, right 0.106 by 0.071 and left 0.101 by 0.074, placed directly opposite each other at posterior end of body. One duct goes forward from each testis and the two

TABLE 4.—Measurements in microns of *Centrocestus armatus*, *C. cuspidatus* and *C. formosanus*

	<i>C. armatus</i> (From Tanabe)	<i>C. cuspidatus</i> (From Looss)	<i>C. formosanus</i> (From Nishigori)	<i>C. formosanus</i> (From Chen, this article)
Body	456 × 236	500 × 350	362 × 197	461 × 212
Oral sucker (length and width)	64 × 68	Diam. 50	50 × 47	60 × 56
Oral spines	44	36	32	30–36
	Size 36 × 19.6	10 × 3	Outer circle: 14 × 2.2 Inner circle: 12.5 × 1.7	Outer circle: 13 × 2 young 19 × 5 old Inner circle: 8 × 2 young 16 × 3 old
Acetabulum (length and width)	50 × 61	Diam. 60	36–43 × 39–55	52 × 55
Pharynx (length and width)	43.8 × 34	30 × 25	40–47 × 20–35	41 × 42
Testes	Right 110 × 60 Left 103 × 60	150 × 80	89 × 43 78 × 42	106 × 71 101 × 74
Ovary	Lobed posteriorly 95 × 65		Lobed 54 × 40	83 × 67
Seminal receptacle	78 × 61	Diam. 70	53 × 50	80 × 62
Seminal vesicle	286 × 46.5	Large	Ant. 122 × 31 Post. 81.2 × 16	Ant. 78 × 35 Post. 69 × 37
Eggs	Number Several tens to over 100	About 50	Over 10 to 45	About 20
	Size 31 × 17	30 × 15 (embryonated)	33 × 17	33 × 16

meet to form large seminal vesicle constricted at about middle into two sausage-shaped parts, posterior portion being 0.037 by 0.069 and going obliquely outward and anterior being 0.035 by 0.078 and going almost transversely inward by following curve of acetabulum. At distal end it narrows considerably and curves upward along right corner of acetabulum, where it joins prostate region on right border of sucker, this portion being surrounded by several unicellular structures, the prostate glands. Terminal portion or ejaculatory duct opens into common genital atrium in front of acetabulum (Fig. 4). In center of the atrium opens genital pore which is transverse slit.

Ovary oval, slightly lobed, measuring 0.083 by 0.067, in front of right or occasionally of left testis. Oviduct passes more or less transversely to middle and receives a short duct from round or oval seminal receptacle, which averages 0.080 by 0.062, and is located in the middle of the body just in front of testes. Oviduct next receives duct from vitelline reservoir and then passes on as an oötype surrounded by Mehlis' glands. Laurer's canal opens apparently into seminal receptacle near junction of oviduct. Uterus bends several times between anterior border of testes and level of genital pore. It contains from 10 to 30 eggs (see Table 2). Metratrum extends upward along curve at left of acetabulum to open into common genital atrium (Fig. 4). Common vitelline duct is just in front of testes and reservoir is somewhat triangular in shape. Vitellaria form spherical follicles located outside of ceca and other organs and extending from lower level of pharynx to region behind testes at posterior end of body.

Eggs show characteristic latticed designs on outside. They measure 17 to 20 μ wide and 32 to 39 μ long with average of 19 by 34 μ . Shape is typically that of chicken egg. Egg shell 1.4 μ thick, uniform throughout; operculum 6 μ wide. Fig. 5 shows most of above characters. Occasionally another type of eggs may be found. These are apparently either first few eggs or are unfertilized. They are narrower and longer, 17 μ by 43 μ , with less conspicuous latticed design (Fig. 6). Egg shell is slightly thinner and often provided with nodule placed slightly to one side at posterior pole of the egg.

Nervous and excretory systems, as far as studied, are as in the metacercaria.

Definitive Hosts: In Formosa: *Nycticorax nycticorax* and dogs, and experimentally in cats, white rats, mice, rabbits, etc. In the Philippines: *Bubulcus ibis coromandus* and *Pyrreroides manilensis*, and experimentally in dogs and cats. In Hong Kong: experimentally in white rats, white mice, chickens, *Mus rattus*, rabbits, ducklings, puppies and kittens.

Location in Host: Small intestine.

NATURAL INFECTION IN RATS AND CATS³

Two worms of this genus were collected from rats, *Mus norvegicus*, in 1933 in Canton but were poorly preserved. Table 4 gives measurements of structures that could be made out from these worms.

The measurements were based on permanent mounts in which, due to some pressure from the cover glass, the size is somewhat exaggerated but the relationships of different organs remain constant. The circumoral spines are distinct but their number cannot be made out. The eggs do not have latticed design on the outside. Because of this and the larger number of eggs the writer is inclined to call this *Centrocestus armatus* tentatively.

The single worm that was collected from a cat in Canton (Chen, 1934) was much like the two from rats and before more can be collected it is placed in the same species.

DISCUSSION

The differentiation of the three known species of *Centrocestus* has been based chiefly on the number of circumoral spines, the form of the margin of the testes and ovary, and the presence or absence of the latticed design on the eggs (see Table 4 for detailed comparison). The number of circumoral spines appears to be rather definite in the three species according to the various authors. Thus in *C. cuspidatus* Looss gave 36 spines, in *C. armatus* Tanabe mentioned 44, and in *C. formosanus* Nishigori counted 32. In the present study, however, it has been shown that in the last species the number of the spines varies from 30–36, with 30–32 being the commonest. In *C. armatus*, Yamaguti in 1933 observed from 40–44 spines and in 1938 only 40. Moreover, it has been noted that spines may easily fall off, and if this happens near the lateral corner of the crown where they are more closely arranged, it will be very difficult for one to detect such an anomaly. These observations indicate that it is not entirely reliable to depend on the number of the spines as a specific character unless a large number of specimens are carefully studied.

With reference to the form of the margin of the testes and ovary it is noted that in *C. cuspidatus* both of these organs have smooth margins while in *C. armatus* and *C. formosanus* they are lobed. In the present study it has been observed that if specimens were left in the physiological salt solution there would be no change in the outline of these two organs, but if they were placed in water the two organs would become smooth. It is well, therefore, to be cautious in considering such characters as of specific importance.

Eggs of the several species are apparently similar in size and shape.

³ Three specimens from these hosts were collected from Canton but all were lost in transit to Hong Kong in the fall of 1938.

Three points, however, require special attention. The first is the latticed design in *C. formosanus*. It is a character which is very important but may be easily overlooked. The question as to whether such a design is present in the eggs of other species cannot be answered, of course, without seeing the specimens. The earlier suggestion that *C. cuspidatus caninus* is a synonym of *C. formosanus* must be verified by a study of the egg shell of the former. The second point of interest is the presence of an almost fully developed embryo in the egg of *C. cuspidatus* according to Looss. This is not true with the two other species. The third point of interest is the number of eggs in a fully matured specimen. Tanabe gave several tens to over a hundred eggs for *C. armatus* and Nishigori gave from over ten to as many as 45 for *C. formosanus*. In *C. cuspidatus* Looss did not mention the number, but from the drawing there are roughly 50 eggs. It is likely that this is not the true number but it shows that they are numerous. Unquestionably the number of eggs depends to a certain extent on the age of the parasite, but this must be within a reasonable limit.

In the present study the age of the fluke as related to the number of eggs present in the body at the time of the autopsy of the host has been subjected to a careful scrutiny. Table 2 shows the result. Among the white rats the number of eggs is over 20 when the parasite is more than 10 days old. Thus in the three flukes of 12 days old, 21, 28 and 32 eggs are found respectively, and in the two flukes of 30 days old, 20 eggs are found in each. In other hosts listed in the table, variations are somewhat greater in the direction of smaller number of eggs. This is particularly true in the 15-day infection in a rabbit in which only four eggs are present in each of the two worms studied, and in the 25-day infection in a mouse in which the number of eggs present in the 9 specimens studied varied from one to 7. Only one worm possesses more than 32 eggs, i.e., the one in a rabbit of 45-day infection in which one of the parasites possesses 48 eggs, the maximum found so far. This must be considered unusual.

This leads us next to a consideration of the definitive host of this parasite. Which is the normal host? The data available from various sources are not sufficient for a positive answer at the present time. As noted in a previous paragraph *Nycticorax nycticorax* and dogs are naturally infected in Formosa, and *Bubulcus ibis coromandus* and *Pyrreroides manilensis* in the Philippines. This does not necessarily mean that they are the optimum hosts. Natural hosts of this parasite in China have not yet been reported. On the basis of experimental data as listed in Table 1 one can readily see that rats are the most favorable hosts as the percentage of worms recovered from each of them is greater than in other hosts. Nevertheless, the number of viable cysts fed to the rats is several times the

number of adults recovered from them. This shows that probably rats are not the optimum hosts.

There is also some indication that a certain amount of acquired immunity may be accumulated, since fewer worms are recovered in infections of longer standing (see Table 2). More intensive study is needed on this phase of the subject.

Attention must also be called to structures like the prepharynx and esophagus which are not only variable but may also be present or absent in specimens depending upon the state of contraction in which they are fixed. In a flattened or relaxed specimen both of these structures are present and sometimes rather long. On the other hand if they are active or killed and fixed when first separated from the host these two structures are either absent or only slightly visible. In a somewhat flattened specimen the prepharynx may be long and slender when the body is extended, or short when the body is contracted. Oftentimes the prepharynx cannot simultaneously contract with the body, but becomes more or less bent to one side forming a pocket. It straightens out immediately as soon as the body is again stretched. Occasionally at the contraction of the body the prepharynx expands on all sides at its lower part so that from ventral or dorsal views it looks as if two prepharyngeal pockets are formed one on each side as seen in the picture of *C. cuspidatus* (Looss, 1896). Due to the varying position of the prepharynx and the pharynx the use of the position of the ganglia as a diagnostic character to distinguish *C. cuspidatus* from *C. armatus* as maintained by Yamaguti will be of no value.

The brief discussion shows that eggs possess clear-cut characteristics and are most dependable for distinguishing the three species. The status of *C. cuspidatus caninus* cannot be settled until its eggs are re-studied. The circumoral spines are important but their variation in number must be borne in mind. Other structures are too variable and cannot be depended on. Table 4 is a compilation of some characters of the various species of *Centrocestus* given by various workers.

The finding of metacercariae of *C. formosanus* in frogs is of considerable importance. From the biological standpoint it shows that heterophyid metacercariae, which have hitherto been known to parasitize only fish in this stage, can also infect other hosts of an entirely different group. The discovery makes it necessary to modify the diagnoses of the family HETEROPHYIDAE Odhner, 1914, and the sub-family CENTROCESTINAE Looss, 1899.

From the public health standpoint the discovery is of additional interest if further work can show that any edible species of frogs, such as *Rana rugulosa*, is involved in this region. Nishigori has already shown that this species is transferable to man. In this region, the only fishes so far involved are small and not ordinarily used as food but if edible

frogs are involved another possible source of human infection exists. The writer has good reason to believe that *R. rugulosa* may be infected and investigation is being carried on in this direction. Considering that frog meat is most delicious when cooked in a "lightning" manner one can easily imagine its potential danger. Perhaps *Centrocestus* in man is overlooked because the parasites are very small and the eggs are similar to commoner species of human flukes, such as *Clonorchis sinensis*.

SUMMARY

The metacercaria of *Centrocestus formosanus* is described in detail for the first time. The parasites encyst chiefly in the gills of four fresh-water fishes, namely, *Macropodus opercularis*, *Puntius semifasciolatus*, *Carassius auratus*, and *Misgurnus anguillicaudatus*, and in the stomach wall and muscles of a common frog, *Rana limnocharis*, and a common toad, *Bufo melanostictus*. The experimentally obtained adult together with its eggs is also treated in detail.

As a result of present observations the eggs are believed to be of importance in specific diagnosis of the three known species. The number of circumoral spines is of value within certain limits because it varies. Other morphological structures appear to be undependable for specific differentiation of the genus.

Worms from natural infections of *Mus norvegicus* and a cat from Canton are believed to be *C. armatus*.

The importance of *C. formosanus* from the public health aspect is briefly discussed.

BIBLIOGRAPHY

- AFRICA, C. M., DE LEON, W. AND GARCIA, E. Y. 1940 Visceral complications in intestinal heterophyidiasis of man. *Acta Med. Philippina* No. 1, pp. 1-132.
- CHAPIN, E. A. 1926 Note on the Heterophyidae. *J. Parasitol.* 12: 180.
- CHEN, H. T. 1934 Helminths of cats in Fukien and Kwangtung Province with a list of those recorded from China. *Lingnan Sc. J.* 13: 261-273.
- FAUST, E. C. 1937 Helminths and helminthic infections. In Craig and Faust's "Clinical Parasitology," Philadelphia, pp. 217-474.
- FAUST, E. C. AND NISHIGORI, M. 1926 The life cycles of two new species of Heterophyidae. *J. Parasitol.* 13: 91-128.
- FUHRMANN, O. 1928 Trematoda, in Kuekenenthal's "Handbuch der Zoologie," Berlin and Leipzig, 2: 1-140.
- LEIPER, R. T. 1913 Seven helminthological notes. *J. London School Trop. Med.* 2: 175-178.
- LOOSS, A. 1896 Recherches sur la faune parasitaire de l'Egypt. I. *Mem. Inst. Egypt* 3: 1-296.
- 1899 Weitere Beiträge zur Kenntniss der Trematoden-Fauna Aegyptens. *Zool. Jahrb. Syst.* 12: 521-784.
- NISHIGORI, M. 1924 On a new species of fluke, *Stamnosoma formosanum*, and its life history. *Taiwan Igakkai Zasshi* No. 234: 181-238. (Japanese, with English summary.)
- PRICE, E. W. 1932 On the genera *Centrocestus* Looss and *Stamnosoma* Tanabe. *J. Parasitol.* 18: 309.

- RANSOM, B. H. 1921 Synopsis of trematode family Heterophyidae with descriptions of a new genus and five new species. Proc. U. S. Nat. Mus. 57: 527-573.
- STILES, C. W. AND C. E. BAKER. 1934 Key-catalogue of parasites reported for Carnivora (cats, dogs, bears, etc.) with their possible public health importance. Bull. Nat. Inst. Health, U. S. Pub. Health Serv., No. 163, p. 945.
- TANABE, H. 1922 Studien ueber die Trematoden mit Suesswasserfischen als Zwischenwirt. 1. *Stamnosoma armatum* n. g., n. sp. Kyoto Igaku Zasshi 19: 1-14. (Japanese, with German abstract.)
- VAZQUEZ-COLET, ANA AND C. M. AFRICA 1938, 1939 Determination of the piscine intermediate hosts of Philippine heterophyid trematodes by feeding experiments. Philippine J. Sc. 65: 293-302; 70: 201-215.
- AND ——— 1940 Morphological studies on various heterophyid metacercariae with notes on the incidence, site, and degree of metacercarial infection in three species of marine fish. Philippine J. Sc. 72: 395-420.
- WITENBERG, A. 1929 Studies on the trematode family Heterophyidae. Ann. Trop. Med. and Parasitol. 23: 131-239.
- YAMAGUTI, S. 1933 Studies on the helminth fauna of Japan. Part 1. Trematodes of birds, reptiles and mammals. Japan. J. Zool. 5: 1-134.
- 1938 Zur Entwicklungsgeschichte von *Centrocestus armatus* (Tanabe) mit besonderer Beruecksichtigung der Cercarie. Z. Parasitenk. 10: 293-296.

EXPLANATION OF PLATE, p. 298

Centrocestus formosanus (Nishigori, 1924)

- FIG. 1. Adult of *Centrocestus formosanus*.
- FIG. 2. Oral sucker of adult showing circumoral spines.
- FIG. 3. Oral sucker of adult showing circumoral spines and rodlets.
- FIG. 4. Acetabular region of adult showing anterior portion of seminal vesicle, prostate region, ejaculatory duct, metraterm, vagina, and genital pore.
- FIG. 5. Egg showing characteristic latticed design of shell.
- FIG. 6. Abnormal egg of unusual length.
- FIG. 7. Encysted metacercaria.
- FIG. 8. Excysted metacercaria.
- FIG. 9. Circumoral spines of metacercaria.
- FIG. 10. Ventral view of excysted metacercaria showing arrangement of skin glands.
- FIG. 11. Excysted metacercaria showing another arrangement of skin glands.
- FIG. 12. Pharynx showing glandular and muscular portions.

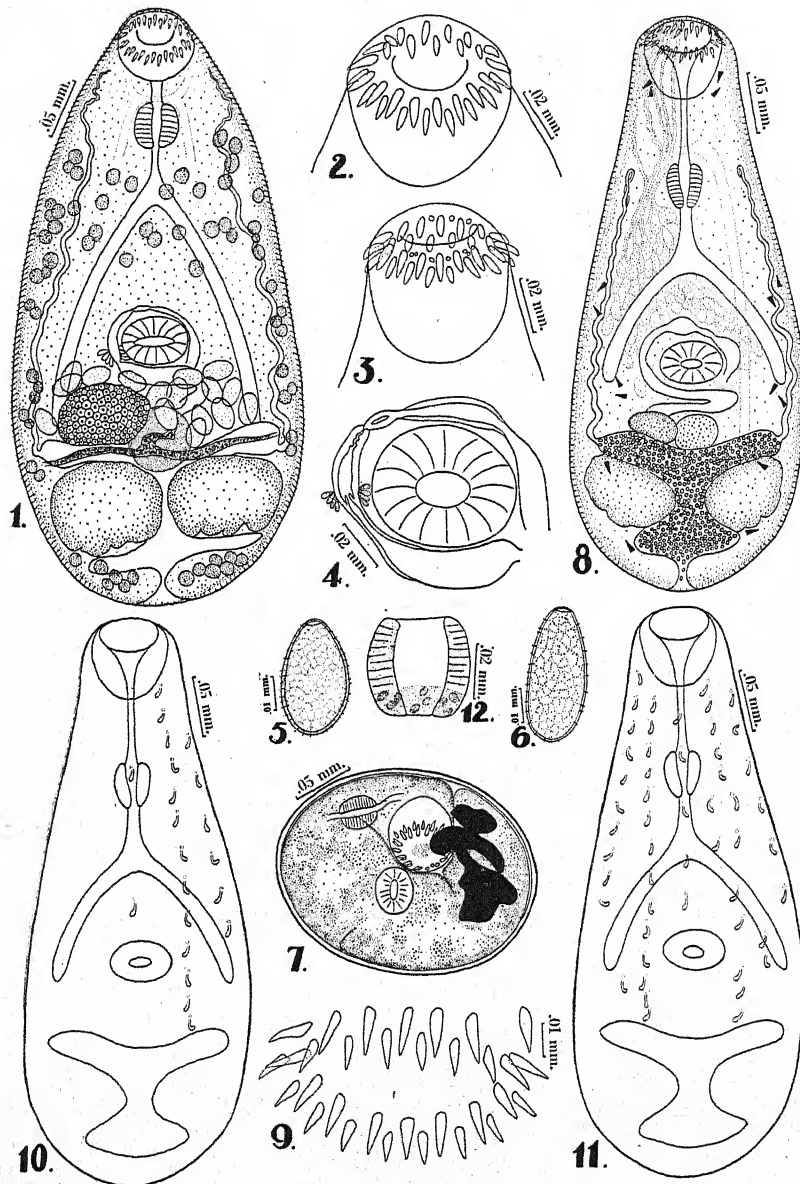


PLATE I

OBSERVATIONS ON THE MACROSCOPIC SPECIES-
IDENTIFICATION OF LARVAL *ANOPHELES*
IN GEORGIA*

R. EDWARD BELLAMY

In most places where routine surveys of local *Anopheles* distribution have been undertaken by large scale collection and identification of larvae from nearby aquatic situations, a certain "familiarity" with the larvae has probably developed. The familiarity, in some cases, has been developed into a technique for distinguishing the larvae of the local species by their macroscopic appearance. Dr. W. V. King, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, related (in lectures to malaria entomology trainees at Orlando, Florida, 1937) that Philippine assistants learned in their country to distinguish macroscopically, with a high degree of accuracy, the larvae of different species of *Anopheles* occurring there. Dr. Arnaldo Gabaldon, Division de Malariologia, Direccion de Salubridad Publica, Ministerio de Sanidad y Asistencia Social, Caracas, Venezuela, stated (in personal communication) that, in Venezuela, sanitary inspectors may learn to distinguish macroscopically the larvae of anopheline species encountered in a small area but that this ability is lost if they are transferred to a new area. Dr. D. Bruce Wilson states (in a manuscript report to the International Health Division of the Rockefeller Foundation, 1940) that in the campaign against *Anopheles gambiae* in Brazil, a technique was developed for distinguishing macroscopically the larvae of this species from larvae of the Brazilian *Anopheles* of the subgenus *Nyssorhynchus*. D. Manson (1934) has tabulated certain macroscopically visible characters of the larvae of eighteen species of Indian *Anopheles* as an aid to the macroscopic identification of these forms, and states that with a short period of training and attention to detail one may learn to distinguish larvae of the more common species of Assam *Anopheles* "with a considerable degree of accuracy."

A few months after inauguration of *Anopheles* survey work in South Georgia, it was noted that the presence or absence of larvae of *Anopheles quadrimaculatus* in collections was usually correctly anticipated in the

Received for publication, October 4, 1941.

* Contribution No. 5 from the Division of Malaria and Hookworm Service, Georgia Department of Public Health, Atlanta, Georgia. Read in part, before the National Malaria Committee and the American Society of Tropical Medicine at Louisville, Kentucky, on November 14, 1940. For their invaluable assistance in connection with this study, I wish to thank Dr. Justin Andrews, Mr. R. S. Howard, Jr., Dr. Glendy G. Sadler, Dr. Horace O. Lund, Mr. Alan Donaldson, and Dr. H. E. Savely, Director, Engineer, and one time Biologists, respectively, of the Division of Malaria and Hookworm Service, Georgia Department of Public Health; and Mr. Melvin H. Goodwin, Jr., Biologist, and Mr. John Platt, Summer Biologist, of the Emory University Field Station, Baker County, Georgia.

field, and that in mixed collections of *A. quadrimaculatus* and *A. crucians* the specimens of the two species could be separated rather successfully "by eye" before the routine determinations by microscope. Larvae of *A. punctipennis* were not found in abundance during this period, and (as microscopic determinations revealed) were "by eye" often mistaken for larvae of *A. quadrimaculatus* and occasionally for larvae of *A. crucians*. Because of the difficulty of distinguishing by eye certain larvae of *A. punctipennis* from certain larvae of the other two common species, it appeared that mistakes in macroscopic identification might be reduced to a minimum if these "difficult" larvae could be separated by eye as such, and only the remainder of the larvae identified macroscopically to species.

After about a year of field experience, a program was inaugurated to check against microscopic determinations the macroscopic identification of all routine collections of fourth-instar *Anopheles* larvae. At the outset of the study, it was decided that each fourth-instar *Anopheles* larva taken in routine work was to be assigned from its macroscopic appearance (and before examination by microscope) to one of the following groups or designations:

- Group 1. Larvae macroscopically identified as *A. quadrimaculatus*.
- Group 2. Larvae macroscopically identified as *A. crucians*.
- Group 3. Larvae macroscopically identified as *A. punctipennis*.
- Group 4. Larvae macroscopically "difficult" but considered from macroscopic appearance to be either *A. quadrimaculatus*, *A. punctipennis*, or *A. walkeri*.
- Group 5. Larvae macroscopically "difficult" but considered from macroscopic appearance to be either *A. crucians*, *A. punctipennis*, or *A. georgianus* (*Anopheles georgianus* was recognized as an entity at the time (Bellamy, 1939) although it was not described until later (King, 1939) and subsequently raised to specific status (King and Bradley, 1941)).

Group 6. Dead and injured fourth-instar larvae.

The "difficult" larvae were to be assigned to groups 4 and 5, but here also a partial separation was attempted in that no provision was made for *A. crucians* in group 4 and none was made for *A. quadrimaculatus* in group 5. As dead and badly injured larvae do not exhibit the characteristics which aid in macroscopic identification, no attempt was made to identify these by eye.

Microscopic determinations to check all macroscopic identifications were planned as routine.

No provision was made for recording the macroscopic identification of *Anopheles barberi*, but with the peculiar appearance and habitat of this species little or no doubt has existed as to the identity of specimens whenever they have been encountered and, as only special collections from

brackish-water areas contained specimens of *A. atropos* and *A. bradleyi*, no provision was made for macroscopic identification of these larvae.

PROCEDURE

The collected fourth-instar larvae of *Anopheles* were brought from the field to the laboratory and there on examination by the unaided eye were assigned to the various groups explained above. The larvae were then placed in individual depressions in a porcelain color plate and were determined by the recognized microscopic characteristics (Bradley, 1936) (chaetotaxy) with a binocular dissecting microscope. On microscopic determination, the larvae were recorded by number of each species under the macroscopic designations (groups) as assigned. In almost all cases the microscopic determinations were accepted as final. Occasional larvae were reared as supplementary check, but in no case was the identification of larva by microscope reversed. During the first year of this study, the collected first-, second-, and third-instar larvae were reared to the fourth larval instar and then treated as the larvae which were fourth instar at first examination of the collections.

MACROSCOPIC IDENTIFICATION OF ROUTINE SURVEY COLLECTIONS

In the preliminary recorded check of macroscopic recognition of the specific identity of larval *Anopheles*, 821 living and 10 dead or injured fourth-instar larvae were examined in 48 collections from as many different ponds and swamps in Calhoun County, Georgia. Almost immediately following the preliminary test in Calhoun County, a similar test was made on collections in Burke County, Georgia, with very similar results obtaining, although the two counties are almost 200 miles apart. Following these tests, only sporadic attention was given to macroscopic identifications. Thus, in a period of more than a year, further macroscopic identifications were checked on only 2460 fourth-instar larvae in 157 collections from 15 different Georgia counties, though several hundred collections were made during this period.

The results of the macroscopic identification of these larvae are presented in Table 1. It may be observed that in the collections from 15 different counties, larvae of *Anopheles punctipennis* predominated and a proportionately higher error was made in the macroscopic identification of larvae in these collections than was made in identification of the Burke and Calhoun County collections in which relatively few larvae of *A. punctipennis* were encountered.

Summary of the Calhoun, Burke, and miscellaneous collections (Table 1) shows that macroscopic identification of 4217 routinely collected fourth-instar anopheline larvae was attempted. Two hundred ninety-nine or 7.1% of these larvae were assigned to the "difficult" groups,

while 3911 (92.9%) were macroscopically identified to species with only a 1.1% error, a macroscopic misidentification of 45 specimens. The error was distributed as follows: of 1570 larvae called *A. quadrimaculatus*, an error of 1.0% (15 specimens) was made; of 1062 larvae called *A. punctipennis*, an error of 2.5% (27 specimens) occurred; and of 1279 larvae called *A. crucians*, an error of 0.2% (3 specimens) was made.

With less than two months of field experience with anophelines, Dr. G. G. Sadler, then a Biologist in the Division of Malaria and Hookworm Service of the Georgia Department of Public Health, attempted macroscopic identification of the 747 fourth-instar larvae taken in his first 70 collections in Burke County, Georgia. The results are presented with his permission and show that the macroscopic identification can be sufficiently learned in a short time to permit a relatively good record in the identification of larval *Anopheles*.

With the exception of one larva which was placed in a "difficult" group, all larvae were assigned by eye to group 1 (*A. quadrimaculatus*), group 3 (*A. crucians*), or group 6 (dead and injured larvae). It is significant that 23 of the 28 *A. punctipennis* which were taken in these collections were recorded in group 6 while only 31 of 377 *A. quadrimaculatus* and 56 of 333 *A. crucians* were placed there. Macroscopic identifications were quite obviously attempted on only those larvae which appeared either to be *A. quadrimaculatus* or *A. crucians*.

In calling 637 larvae from their macroscopic appearance, Dr. Sadler made an error of less than 6.5% although his previous experience with anophelines consisted of only three weeks of study with the writer and another month of individual study. The identifications are analyzed in Table 1.

IMPORTANCE OF KNOWING THE HABITAT FROM WHICH LARVAE WERE COLLECTED

There is little doubt that knowledge of the field conditions obtaining where the various collections of larvae on which the above tests of macroscopic identification were made was of considerable assistance toward the correct macroscopic identification of the larvae. In order to evaluate the significance of the habitat-association factor, seven malaria workers (Dr. H. O. Lund, Dr. H. E. Savely, Dr. G. G. Sadler, and Mr. Alan Donaldson, Summer Biologists, and Mr. R. S. Howard, Jr., Malaria Investigations Engineer, all of the Division of Malaria and Hookworm Service, Georgia Department of Public Health; and Mr. M. H. Goodwin, Jr. and Mr. John Platt, Biologist and Summer Biologist, respectively, of the Emory University Field Station, Newton, Georgia) variously experienced in malaria entomology, assisted the writer in a wholesale collection of anopheline larvae. The men separated into four groups and the col-

TABLE 1.—Distribution (determined by microscope) of macroscopically identified 4th-instar larval Anopheles

	"Definite" groups												Proportion assigned to groups 1, 2, & 3.	Error made in groups 1, 2, & 3.	"Difficult" groups						Total larvae				
	Group 1 (<i>A. quad.</i>)				Group 3 (<i>A. punc.</i>)				Group 2 (<i>A. c. cruc.</i>)						Group 4			Group 5				Group 6 Dead larvae			
	<i>quad.</i>	<i>punc.</i>	<i>cruc.</i>	<i>walk.</i>	<i>punc.</i>	<i>quad.</i>	<i>cruc.</i>	<i>walk.</i>	<i>cruc.</i>	<i>quad.</i>	<i>punc.</i>	<i>walk.</i>			<i>cruc.</i>	<i>quad.</i>	<i>punc.</i>	<i>georg.</i>	<i>quad.</i>						
<i>Routine collections</i>																									
Calhoun County 48 collections	532	2	1	0	70	0	3	0	173	0	0	0		95.1%	15	7	0	3	11	1	0	3	10	831	
Burke County 55 collections	422	0	0	0	45	0	0	0	417	0	1	0		94.5%	19	12	1	7	11	1	0	0	70	1006	
157 collections from 15 counties . .	601	12	0	0	927	17	7	0	686	1	1	0		90.8%	79	75	0	8	28	9	8	1	510*	2970	
Totals of above	1555	14	1	0	1042	17	10	0	1276	1	2	0		92.9%	113	94	1	18	50	11	8	4	590	4807	
70 Burke colls. (G. G. Sadler)† . . .	340	4	20	9	0	0	0	0	257	1	5	0		85.3%	0	0	0	0	0	0	0	1	110	747	
<i>Mixed Series</i>																									
Macroscopically identified by:																									
The writer	403	8	10	0	280	14	2	1	308	3	0	1		97.8%	8	3	1	3	5	1	0	2	0	0	1053
Subject No. 1	406	63	63	1	224	18	42	0	221	6	2	2		99.5%	1	2	0	2	0	0	0	0	0	0	1053
Subject No. 2	320	55	9	1	194	55	95	0	220	33	32	2		96.5%	20	11	0	2	2	2	0	0	0	0	1053

* Includes (with dead larvae) collected young instar specimens which were reared to fourth instar and then identified by microscope only.

† Since Dr. Sadler lumped all "difficult" larvae with dead larvae in Group 6, all larvae in Group 6 have been scored as "difficult" in computing percentage assigned to first three groups; elsewhere Group 6 has been ignored.

lections were made at varied habitats in four different counties in order that there might be, as nearly as possible, an equal and diversified representation of the three common species. The larvae were collected on two successive days and, when brought to the laboratory, were thoroughly mixed.

After completion of these collections, a person inexperienced in macroscopic identification of anopheline larvae pipetted representative fourth-instar specimens individually from the assorted lot to numbered depressions in porcelain color plates. Eight plates were used and each plate had twelve depressions; thus, with one larva in each depression, 96 specimens were available at one time for macroscopic identification. Working independently, the writer and the three of the malaria workers mentioned above who had had some experience in the techniques of macroscopic identification applied themselves to the identification and recording of these sets of larvae by the unaided eye, while the other workers, as the plates of larvae were rotated to them, determined the same larvae by microscope and recorded the results. The macroscopic identifications were checked against the determinations by microscope after all the identifications were complete on the series of 96 larvae. These specimens were then discarded and a second series of larvae was similarly selected, identified, and checked. In this manner eleven series of larvae, totalling 1056 specimens, were checked. The four malaria workers who had had little or no experience in the techniques of macroscopic identification alternately attempted macroscopic identification of about 200 larvae each.

According to the determinations by microscope, the 1056 fourth-instar larvae consisted of 328 *A. crucians*, 427 *A. quadrimaculatus* (including 2 doubtful specimens), 295 *A. punctipennis* (including 12 doubtful specimens), 3 *A. walkeri*, and 3 specimens which were either *A. quadrimaculatus* or *A. punctipennis*. These last three failed to rear to adults as check and were scored as follows: if in the macroscopic identifications they were designated either as *A. quadrimaculatus* or as *A. punctipennis* they were subtracted from the total and were not considered—otherwise they were counted in the total as mistakes.

The writer assigned 2.2% of the larvae to difficult groups and made a 3.8% error in the macroscopic identification of the other 1030 specimens (a misidentification of 39 larvae). The errors made by the other workers ranged from 18.8% to 55.3%. The analysis of these macroscopic identifications (for the writer and two other workers) is shown in Table 1.

The fact that the error made in the macroscopic identification of the mixed series of larvae (3.8%) was greater than that made in identification of routine field collections indicates that the habitat-association factor is of some assistance to correct macroscopic identification. The charac-

teristics of service in macroscopic identification of larvae, however, are most distinct when specimens are in the field or have only recently been brought from the field (a fact also observed by Manson (1934)), and some of the specimens in the mixed series had been collected two days before macroscopic identification of them was attempted. This must account in part for the higher error in the identification of the assorted larvae.

MACROSCOPIC IDENTIFICATION OF PUPAE AND THIRD-INSTAR LARVAE

Immediately following the macroscopic identification of the 1056 fourth-instar larvae, all third-instar larvae which were present in the large mixture of collections were separated by eye into three groups, were placed in three jars, marked "*A. quadrimaculatus*," "*A. punctipennis*," and "*A. crucians*," respectively, and were fed yeast; no specimens were assigned to "difficult" groups. All of the pupae present in the mixture of collections were similarly sorted into three jars marked like those for the third instar larvae; provision was made for the retention of adults as they emerged. Subsequently, Mr. M. H. Goodwin, Jr. identified the pupae as emerged adults and the larvae by microscope as the latter transformed to fourth instar. According to Mr. Goodwin's observations, the results of the macroscopic identification of the third-instar larvae were as follows:

Of the 115 third-instar larvae macroscopically designated as *A. quadrimaculatus* 70 reared to fourth-instar *A. quadrimaculatus*, 5 to fourth-instar *A. punctipennis*, and 2 to fourth-instar *A. crucians*, while 2 were unidentified and 36 died as third-instar.

Of the 88 third-instar larvae macroscopically designated as *A. punctipennis* 72 reared to fourth-instar *A. punctipennis*, 1 to fourth-instar *A. quadrimaculatus*, and 3 to fourth-instar *A. crucians*, while 12 died as third-instar larvae.

Of the 186 third-instar larvae macroscopically designated as *A. crucians* 70 reared to fourth-instar *A. crucians*, 2 to fourth-instar *A. punctipennis*, and none to fourth-instar *A. quadrimaculatus*, while 4 were unidentified and 110 died.

If the larvae which died as third-instar are disregarded and the six larvae which were not identified by microscope are counted as mistakes, an 8.2% error was made in the macroscopic identification of 231 third-instar larvae from the assorted lot; if the six unidentified larvae are disregarded along with those which died as third-instar, the error is less than 5.8%.

From the pupae, Mr. Goodwin identified reared adults as follows:

Pupae macroscopically identified as *A. quadrimaculatus* reared to 88 *A. quadrimaculatus*, 14 *A. crucians*, and 13 *A. punctipennis* adults.

Pupae macroscopically identified as *A. punctipennis* reared to 25 *A. punctipennis*, 21 *A. quadrimaculatus*, and 31 *A. crucians* adults.

Pupae macroscopically identified as *A. crucians* reared to 36 *A. crucians*, 13 *A. quadrimaculatus*, and 9 *A. punctipennis* adults.

The error in the attempted macroscopic identification of pupae was 40.4%, somewhat better than chance but an exceedingly large error.

Previous checks of macroscopic identification of pupae and third-instar larvae gave similar but much better results in the case of the pupae and somewhat better results in the case of the third-instar larvae. However, in these earlier checks knowledge of the identity of the fourth-instar larvae in the collections with the pupae and third-instar larvae undoubtedly served as a valuable guide to the correct guessing of the identity of these latter.

While the experience in macroscopic identification has largely consisted of identification of collections of larvae from southwest Georgia counties, the macroscopic method has been checked against collections of anophelines from Georgia counties bordering the Atlantic Ocean, South Carolina, Tennessee, Alabama, and Florida. No significant difference was noted in the macroscopic appearance of larvae from these different counties.

CHARACTERISTICS EMPLOYED IN MACROSCOPIC IDENTIFICATION

The Table of Characteristics (Table 2) given here is the result of impressions from three years of observations of *Anopheles* larvae and does not represent results of tabulations of numbers of specimens of the different species in the different color phases, etc. Indeed, the gradations in coloration, arrangement of the white dorsal spotting, and the other characteristics would apparently form such a complete series of varieties that an attempted classification on which to base a tabulation of specimens would be exceedingly difficult to establish or, once established, to follow.

As already indicated, a knowledge of the type of habitat from which larvae are taken helps much in making decisions as to their identity. Most helpful of all, certainly, is familiarity with the larvae—the more different “macroscopic types” of larvae with which one is familiar, the better prepared will he be to identify macroscopically a given collection of larvae.

In a tabular analysis of diagnostic macroscopic characters of the larvae of the more common *Anopheles* species of Assam, Manson (1934) uses general body coloration, general body size, color and size of head, pattern of spotted markings on dorsum, and degree of serration of abdominal outline.

No mutually exclusive characteristics have been found which will serve to distinguish macroscopically *all* of the larvae of any of the common Georgia species of *Anopheles* from *all* the larvae of any other species. However, some specimens of each species exhibit characteristics which

are considered diagnostic, and on a combination of characteristics, many specimens can be given a macroscopic identification sufficiently reliable to leave little doubt as to its correctness.

In some cases it has been rather difficult to decide just what macroscopic characteristics have been employed in the separation of larvae of different species when the macroscopically visible differences between

TABLE 2.—Table of macroscopic characteristics of 4th-instar *Anopheles* larvae

	<i>A. quadrimaculatus</i>	<i>A. punctipennis</i>	<i>A. crucians</i>
General coloration	Majority yellow, green, or greenish; many dark brown or black. Head of most light-colored specimens orange red, paler posteriorly (such specimens usually have broad light area involving anterior margin of thorax thus continuing posterior "paling out" of head coloration.	Majority dark brown or black; some light green; (specimens from shallow, turbid puddles usually grayish). Light specimens with reddish head not uncommon but head of these rarely lighter posteriorly or bordered by light coloration on anterior margin of thorax.	Majority dusky brown, dark brown, or dark green. Some dull green specimens with reddish head but head of these not lighter posteriorly and rarely if ever bordered by white area on anterior margin of thorax.
Presence of dorsal linear white markings	Often present, especially on yellowish and dark specimens.	Sometimes present; grayish specimens from turbid puddles almost all with some dorsal pattern.	Often present but to minimum extent on most specimens.
Pattern of white or light dorsal linear markings	Arranged as a central linear band or segments of such a band. In many specimens band widens anteriorly on thorax to form broad triangle with base along anterior thoracic margin. At abdominal segments 3 and 5 light color concentrations diamond shaped. (Rarely, light spotting on a specimen limited to a pair of whitish marks on abdominal segments 3 or 5—although a dark area may separate these marks, they do not lie parallel but can be seen to represent the anterior margins of a more or less obscured "diamond spot").	Arranged as a central linear band or segments of such a band. Whitish coloration widening anteriorly on thorax to form broad triangle in very few specimens. Light color concentrations at abdominal segments 3 and 5 diamond shaped (bicolorous "diamond spots" characteristic of grayish specimens from turbid puddles).	Characteristically arranged in two, usually thin, parallel dorso-linear stripes or paired segments of the stripes. Whether white coloration consists of continuous lines, several paired segments of the lines, or a mere pair of light parallel dashes on abdominal segment 3 or 5, a narrow, central linear dark area separates the coloration (in almost all specimens) into bilaterally symmetrical halves. (Any white coloration not centrally divided consists of segments squared at the ends—never "diamond shaped.")
Activity	Variously lively or lethargic in feeding and swimming at water surface, but rarely "nervous."	Characteristically "nervous," exhibit rapid feeding motions, and move quickly and frequently from one place to another (particularly when confined in a container).	Characteristically lethargic not lively in swimming along water surface or in feeding motions.
"S"-shaped flexure at water surface	Majority of specimens, while at water surface, will contract body into "S" shape—especially if container in which they are placed is gently agitated. ("S"-shaped flexure usually relaxed after about one minute.)	Many specimens, while at water surface, will contract body into "S" shape—especially if container in which they are placed is gently agitated.	Specimens never exhibit "S"-shaped flexure (occasionally specimens raking mouth brushes on comb of eighth abdominal segment or turning head under abdomen nearly simulate "S"-shaped flexure).

TABLE 2.—(Continued)

	<i>A. quadrimaculatus</i>	<i>A. punctipennis</i>	<i>A. crucians</i>
Relation to surface film	Specimens may be dislodged from contact with surface film by sharp blows on container in which they are placed. Most dislodged specimens apply mouth parts to spiracular plate while sinking downward of own body weight; some swim downward voluntarily; others return more or less abruptly to surface.	Specimens are dislodged from contact with surface film with much difficulty, however, frightened specimens usually release contact with surface film and swim to bottom voluntarily—and often abruptly back to surface without resting at bottom (specimens which lose contact with surface film will rarely sink to bottom of own body weight).	Specimens are easily dislodged from contact with surface film by sharp blows on container in which they are placed. Usually dislodged specimens assume a rigor with anterior and posterior ends bowed dorsally to form a rough arc or crescent and in this condition sink downward of their own body weight. Specimens only rarely swim forcibly to bottom when disturbed in a container.
Head outline and angle of antennae	Head appears large and square; antennae apparently make at least 45-degree angle with longitudinal axis.	Head appears small and rounded; antennae usually not conspicuous.	Head appears rather "egg-shaped," elongate, and broader posteriorly than anteriorly. Antennae apparently make less than 45 degree angle with longitudinal axis.
Thoracic outline	Thoracic outline (especially "shoulders") appears squared.	Thoracic outline (especially "shoulders") of most specimens appears rounded.	Thoracic outline appears oblong without markedly abrupt right angles at "shoulders."
Appearance of abdomen (viewed in dorsal aspect)	Margins of abdomen appear quite serrate.	Margins of abdomen appear moderately serrate.	Margins of abdomen appear as series of evenly rounded humps.
Size and proportions	Many specimens large and robust.	Many specimens rather small and delicate.	Most specimens of average size; larger specimens appear elongate and loosely constructed.

them have appeared to be poorly defined. As differences that may seem to be of a rather trivial nature have been employed in some such cases and in a supplementary capacity quite often, these differences have been included in the Table of Macroscopic Characteristics. Characteristics are given in the Table as typical of *most* larvae of a given species when some larvae of that species have been observed which have not exhibited these characteristics; in fact, certain specimens possess some attributes that are considered typical of larvae of one species and other attributes that are characteristic of larvae of another—these are the larvae that in practice have been considered "difficult," and identification of these cannot be successfully effected without resort to the microscope unless more reliably diagnostic characteristics can be discovered. The mistakes that have been made in the macroscopic identifications (as shown in Table 1) have probably resulted in part from an occasional hasty or careless application of the macroscopic criteria to specimens to be identified but in other cases represent exceptional specimens of one species which exhibited a preponderance of the characteristics which are considered to be indicative of another species. In general, the larvae of *Anopheles crucians* and *A.*

georgianus seem to have less in common with larvae of *A. quadrimaculatus*, *A. punctipennis*, and *A. walkeri* than do these latter with each other.

The reference to color in the Table of Characteristics as of some diagnostic assistance is based on the rough proportions of larvae of each species that have been observed in the various listed colors or shades of color since each color has been found represented by at least a few larvae of each species. The nature of the dorsal white-spotted coloration of specimens thus spotted is of more diagnostic value than general body color, especially for separating larvae of *A. crucians* from those of the other species. Boyd (1930, p. 203) notes that this spotting on anopheline larvae has been considered diagnostic by some, and Manson (1934) finds these markings of assistance in distinguishing larvae of Indian *Anopheles* in Assam. Some larval specimens of each species exhibit this pattern of central dorso-linear white-spotting which may either be in the form of a continuous stripe (or paired stripes) or in the form of one or more segments or portions of the stripe. When limited to segments of the stripe, these will be at abdominal segment 3, abdominal segment 5, or on the thorax, and in specimens which have the complete stripe, any concentrations or broad areas in the stripe will be at one or more of these points.

MACROSCOPIC CHARACTERISTICS OF 4TH-INSTAR LARVAE OF THE LESS COMMON SPECIES

Too few specimens of the larvae of *A. walkeri* and *A. georgianus* have been observed since the significance of macroscopic appearance was recognized to allow for a very complete analysis of the various macroscopic characteristics of these larvae. In general, the larvae of *A. walkeri* appear more similar to larvae of *A. quadrimaculatus* than to those of the other species. The dorsal white coloration on the spotted specimens consists, as in *A. quadrimaculatus* and *A. punctipennis*, of a central linear band or portions of the band, but on many specimens has been noted to branch anteriorly on the thorax to form a narrow-armed "Y." At the water surface, specimens often contract into an "S" shape as do *A. quadrimaculatus* and *A. punctipennis*. In shape of head and body outline and proportions, most specimens appear similar to larvae of *A. crucians* although some specimens appear more similar to larvae of *A. quadrimaculatus* in these respects.

Larvae of *A. georgianus* appear generally similar to larvae of *A. crucians* although those of the former species seem to exhibit less white coloration dorsally and to be more inclined to swim voluntarily to the bottom when disturbed.

SUMMARY

Following a period of relatively intense study in Georgia of the macroscopic appearance of fourth-instar larvae of *Anopheles quadrimacu-*

latus, *A. crucians*, and to a lesser extent of *A. punctipennis*, Georgia specimens of these species were distinguished without magnification with a rather high degree of accuracy. A small percentage of specimens were found to present an appearance which made decisions as to their identity difficult. After two years of experience with the macroscopic characteristics of the larvae of Georgia anophelines, an error of nearly 4% was made in the attempted macroscopic identification of over a thousand larvae consisting of a mixture of nearly equal numbers of specimens of the three common species. Third-instar specimens were distinguished with a slightly higher percentage of error.

The fact that no table of mutually exclusive macroscopic characteristics has as yet been prepared which will serve satisfactorily to separate the larvae of the three species and that an attempted analysis of the characteristics employed in such a separation is largely based on relative appearance, indicates that (for the present at least) identification of the larvae by eye cannot be made a reliable substitute for the commonly employed identification by microscope that is based on the larval chaetotaxy. However, an ability to recognize the majority of larvae of the several species when encountered in the field can be achieved with practice, and may be employed to much advantage in studies of the associations of the larvae of each species with various aquatic "microhabitats." Thus when larvae are collected from a variety of different vegetations and other habitats in a single pond, subsequent discovery by microscopic determinations alone that three species are represented in the collection will not reveal much as to the exact situations from which specimens of the different species were taken; while a macroscopic field recognition of these larvae as the collecting proceeded would reveal the species of the larvae present in each type of vegetation or other habitats and the relative proportions in which they occur in each; subsequent determinations by microscope would check the identifications made in the field. Also, it is often a distinct advantage in practical survey work to know while in the field, and with a fair degree of certainty, of just what species a given collection of larvae may consist.

BIBLIOGRAPHY

- BELLAMY, R. E. 1939 An anopheline from inland Georgia resembling the brackish-water race of *Anopheles crucians*. J. Parasitol. 25: 186.
- BOYD, MARK F. 1930 An Introduction to Malariaology. Cambridge, Mass. 437 pp.
- BRADLEY, G. H. 1936 On the identification of the mosquito larvae of the genus *Anopheles* occurring in the United States. South. Med. J. 29: 859-861.
- KING, W. V. 1939 Varieties of *Anopheles crucians* Wied. Am. J. Trop. Med. 19: 461-471.
- KING, W. V. AND G. H. BRADLEY 1941 General morphology of *Anopheles* and classification of the Nearctic species; pp. 63-70 in "A Symposium on Human Malaria," Washington, D. C. 398 pp.
- MANSON, D. 1934 Some notes on the identification of some anopheline larvae by macroscopic methods. Rec. Malar. Surv. India, 4: 197-203.

A PARASITE OF THE MACRONUCLEUS OF *VORTICELLA*

HAROLD KIRBY

Department of Zoölogy, University of California, Berkeley

In a sample of water from each of two water troughs used by horses at the Frances Simes Hastings Natural History Reservation, Monterey County, California, many specimens of *Vorticella* were found with the macronucleus parasitized by bacteria. By comparison with the taxonomic accounts by Noland and Finley (1931) and Kahl (1935) the species of ciliate seemed to be *V. similis* Stokes. I am indebted to Dr. J. M. Linsdale, Director of the Reservation, for the collections of water containing the ciliates. The names of the troughs in the following paragraph are those used by him in the geographical records of the Reservation.

The first sample was obtained from Upper Barn Trough on February 5, 1941. Upon examination of floated coverglasses on the following day I found numerous specimens of *Vorticella*, and all of them had parasitized macronuclei. Those observed on the next day also were all parasitized, but the number had greatly decreased. The ciliate was present in a collection made from the same trough on February 13, but no nuclear parasites were found. The second sample with parasitized *Vorticella* was obtained from Cow Pasture Trough on March 29, 1941; in this material there were also many unparasitized specimens.

In the living ciliates, the hypertrophied nuclei provided an indication of the presence of the parasites, which on close examination could easily be seen as small refractile rods (Fig. 3). One specimen with a parasitized nucleus was seen in fission, with the macronucleus in a dumb-bell form. Fission was completed while the specimen was watched. Detailed studies of the parasite were made in Schaudinn-fixed, iron haematoxylin-stained preparations.

When not parasitized, the greatly elongated macronucleus has a varying width that often is about 4-5 μ . Nuclei only a little thickened, and with few scattered parasites, were seen. In many instances the parasitized macronucleus had a width of 10, 13, or more microns, usually retaining its elongated form. At an extreme of hypertrophy, it had become a large ellipsoidal body measuring about $\frac{3}{4}$ the length of the ciliate and $\frac{1}{4}$ of its width. In one specimen the parasitized macronucleus had fragmented into two rounded bodies, one with a diameter of 23 μ and the other of 15 μ .

There is a marked alteration of the substance of the macronucleus even when the parasites are present in small numbers. Nuclei not parasitized contain numerous close-set, deep-staining, relatively large granules

Received for publication, October 8, 1941.

of chromatin (Fig. 1). In parasitized nuclei on the same slides the macronuclear substance is gray, and its texture at moderate magnification seems about homogeneous. At the highest magnification it may be seen to be alveolar or obscurely granular, but there is no sharp resolution of structure like that of the normal nucleus.

The parasites are deep-staining rods, straight or slightly curved in

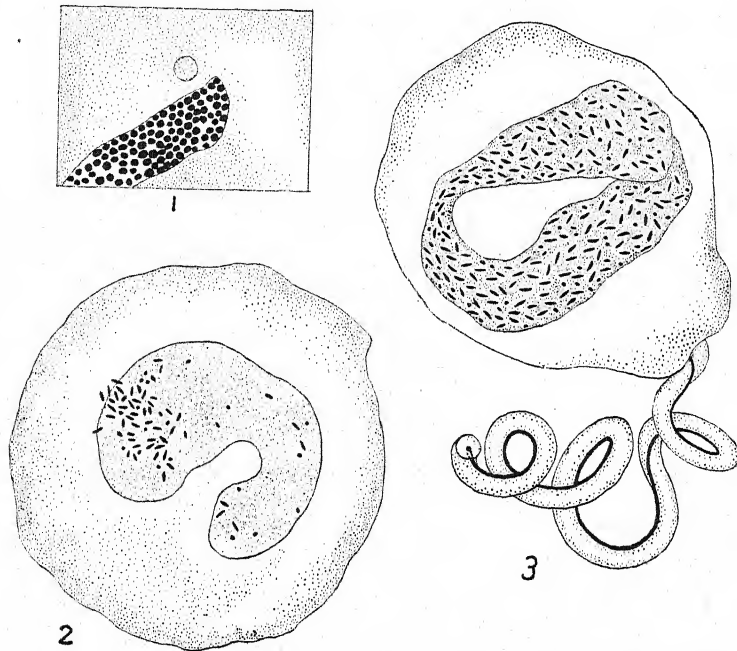


FIG. 1. A portion of the normal macronucleus of *Vorticella*, showing deep-stained chromatin granules.

FIG. 2. Macronucleus of *Vorticella* parasitized by bacteria, and hypertrophied most in the region where there are most parasites. A few bacteria of the same form are in the cytoplasm just outside the macronucleus.

FIG. 3. Macronucleus heavily parasitized with evenly distributed bacteria. All the parasites are fusiform; those that appear as granules are represented as seen from the end.

All figures $\times 1275$. Fixation Schaudinn. Stain Heidenhain's iron haematoxylin.

In preparation of these figures, assistance rendered by personnel of Work Projects Administration, Official Project 65-1-08-113, Unit C 1, is acknowledged.

form, and homogeneous in so far as demonstrated (Fig. 3). Their length ranges from about 0.5μ to 1.5μ . Some of the rods have pointed ends and a neat spindle form. Others are rounded at one or both ends. Each rod lies in the substance of the macronucleus in a vaguely defined clear area. They are oriented in all directions, and in most instances observed were uniformly distributed throughout the macronucleus. In several specimens the parasites were for the most part grouped in one region of

the nucleus. This part of the nucleus was more greatly enlarged than the part with few parasites. Occasionally a few parasites were found to lie outside the macronucleus, but in contact with it (Fig. 2).

Numerous vorticellas with parasitized nuclei were studied, and in none were there rods of larger size than those described above.

Parasites of the macronucleus of ciliates have been recorded by many authors. The most frequently reported parasite is of the *Holospora* type. This organism was observed in *Paramecium caudatum* by Müller (1856), Balbiani (1861), and many others. (See review by Kirby, 1941.) Recently it has been studied by Wichterman (1940). It may reach a length surpassing $20\ \mu$; Fiveiskaja (1929) reported a maximum of $30\ \mu$. There are stages in development that are spindle-shaped and only $3\text{--}6\ \mu$ long, but these develop rapidly into the larger forms (Fiveiskaja). Stein (1867) reported threads much like those in the nucleus of *Paramecium* in *Pleuronema chrysalis* and *Enchelyodon farctus*.

Parasites of smaller size were found in macronuclei of *Chilodon cucullulus* ($4\ \mu$ long, according to Engelmann, 1862); *Blepharisma lateritia* (Engelmann, 1862); *Stentor roeselii* ($3.7\text{--}5.6\ \mu$, Stein, 1861, 1867); and *S. polymorphus* ($6\ \mu$, Balbiani, 1893). These parasites had in common differentiation of at least some forms for part of the length into a denser, more refractile substance, with the remainder a clearer substance. This differentiation is characteristic of *Holospora*. Engelmann (1876) reported in *Stylonychia mytilus* enlarged bodies that seemingly were nuclei or nuclear fragments, which were densely infested with cylindrical or biscuit-formed bacteria at most $3\ \mu$ long, ranging down to $\frac{1}{2}$ this size. The same bacteria, he stated, were abundant in the water.

I have found no record of parasites of the nuclei of species of the genus *Vorticella*. In the taxonomic accounts by Noland and Finlay (1931) and Kahl (1935) nuclear parasites are not mentioned. The authors must have observed a great deal of material, and their failure to record the parasites must indicate that they are not a common occurrence. For other peritrichs, there seems to be only the report by Greeff (1871) of rods in the macronucleus of *Epistylis flavicans*. He recognized that these structures were similar to those previously described in *Paramecium*.

A unique macronuclear parasite was found in *Prorodon* by Stein (1867), Švec (1897), and Kahl (1930). Stein noticed in the unenlarged macronucleus of *Prorodon teres* spindle-shaped bodies parallel to one another and attaining $36\ \mu$ in length. Švec named a new species, *P. nucleatus*, the macronucleus of which contained 4 or 5 large rods. Kahl stated that similar rods are found at times in various species of *Prorodon*, and must be regarded as parasites.

So far as I have been able to learn, the minute bacteria that I have observed in the macronucleus of the species of *Vorticella* are not the same as any nuclear parasite hitherto described. The parasite causes structural alteration and hypertrophy of the macronucleus.

BIBLIOGRAPHY

- BALBIANI, E.-G. 1861 Recherches sur les phenomenes sexuels des infusoires. J. Physiol. Homme et Animaux 4: 102-130; 194-220; 431-448; 465-520.
 ——— 1893 Nouvelles recherches experimentales sur la mérotomie des infusoires ciliés. Ann. Microg. 5: 1-25.
- ENGELMANN, T. W. 1862 Zur Naturgeschichte der Infusionsthiere. Z. Wissensch. Zool. 11: 347-393.
 ——— 1876 Ueber Entwicklung und Fortpflanzung von Infusorien. Morphol. Jahrb. 1: 573-635.
- FIVEISKAJA, A. 1929 Einfluss der Kernparasiten der Infusorien auf den Stoffwechsel. Arch. Protistenk. 65: 275-298.
- GREEFF, R. 1870, 1871 Untersuchungen über den Bau und die Naturgeschichte der Vorticellen. Arch. Naturg. 36 Jahrg. 1: 353-384. 37 Jahrg. 1: 185-221.
- KAHL, A. 1930 Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 1. Allgemeiner Teil und Prostomata. In Dahl: "Die Tierwelt Deutschlands," Jena. 18. Teil.
 ——— 1935 Idem 4. Peritricha und Chonotricha, Ibid. 30. Teil.
- KIRBY, H. 1941 Organisms living on and in protozoa. In Calkins and Summers: "Protozoa in Biological Research," Columbia Univ. Press, New York.
- MÜLLER, J. 1856 Einige Beobachtungen an Infusorien. Monatsb. K. Preuss. Akad. Wissensch. 1856: 389-393.
- NOLAND, L. E. AND FINLEY, H. E. 1931 Studies on the taxonomy of the genus *Vorticella*. Tr. Am. Micr. Soc. 50: 81-123.
- STEIN, F. (RITTER) VON 1861 Über die Conjugation der Infusionsthiere und über die geschlechtliche Fortpflanzung der Stentoren. Sitzungsber. K. Böhm. Gesellsch. Wissensch. 1861: 62-77.
 ——— 1867 Der Organismus der Infusionsthiere. II. Abth. (1) Darstellung der neusten Forschungsergebnisse über Bau, Fortpflanzung und Entwicklung der Infusionsthiere. (2) Naturgeschichte der heterotrichen Infusorien. Leipzig.
- ŠVEC, F. 1897 Beiträge zur Kenntnis der Infusorien Böhmens. I. Die ciliaten Infusorien des Unterpočernitzer Teiches. Česka Akad. Fr. Jos. Prag. (Math-Nat.) 4: 29-47.
- WICHTERMAN, R. 1940 Parasitism in *Paramecium caudatum*. J. Parasitol. 26 Suppl.: 29.

FURTHER STUDIES UPON THE CHEMISTRY OF *MACRACANTHORHYNCHUS HIRUDINACEUS*

THEODOR VON BRAND AND JEAN SAURWEIN

Department of Biology, The Catholic University of America, Washington, D. C., and
Department of Chemistry, Barat College, Lake Forest, Ill.

In two previous papers (von Brand, 1939, 1940) some aspects of the chemical composition of *Macracanthorhynchus hirudinaceus* were discussed. The present study is an extension of these investigations and deals with the polysaccharides and some inorganic constituents occurring in the worms.

THE POLYSACCHARIDES

As stated previously (von Brand, 1939) a polysaccharide can be isolated from these parasites in fairly large quantities by Pflueger's procedure. It gives typical glycogen reactions (opalescence of the aqueous solution, deep brown-red color with iodine, sugar identified as glucose after hydrolysis). However, upon digestion with filtered saliva, a small indigestible fraction was found which gave no color reaction with iodine and reduced Fehling's solution only after hydrolysis with hydrochloric acid. Since it was clearly not glycogen, the only polysaccharide so far described from any parasitic worm, it seemed of interest to extend these observations.

The crude polysaccharide was isolated from about 500 gm of freshly collected worms by Pflueger's method. It was further purified by heating for two hours on a boiling water bath with 60% KOH, following which it was precipitated with alcohol once from alkaline, once, after filtration, from acid and four times from neutral aqueous solution. It was then washed repeatedly with 96% alcohol, 100% alcohol, and ether, and the whole purification process repeated once more. The yield was 4.5 gm of a pure white powder, readily soluble in water with strong opalescence. It was nitrogen-free (no Prussian blue precipitate was obtained on treatment of the filtrate of the sodium fusion of the polysaccharide with ferrous sulfate, ferric chloride and hydrochloric acid), and it was practically ash-free.

Of this substance, 200 mg were dissolved in 25 ml of distilled water and the optical rotation determined in a 1-dm tube.

25° C; Na light (D)

Zero reading = -0.07°

Optical rotation = +1.43° - (-0.07°) = +1.50°

$\alpha \frac{20}{D} = \frac{150^\circ \times 25}{0.2} = +187.5^\circ$

Received for publication, October 8, 1941.

This is somewhat less than the most reliable figure for glycogen ($\alpha \frac{20}{D} = +196.6^\circ$, according to Gatin-Gruzewska, 1904).

The material was then digested for 24 hours with filtered saliva at 37°C , bacterial growth being prevented by addition of a few drops of toluene to the solution. The indigestible fraction was isolated by precipitation with alcohol. After reprecipitation it was heated for two hours on a boiling water bath with 60% KOH, precipitated with alcohol one time each from alkaline, acid and neutralized aqueous solution. It was then digested again, and the purification process outlined above was repeated.

A small amount of white material resulted which gave an opalescent aqueous solution. It was hydrolyzed with dilute hydrochloric acid. To a drop of the hydrolyzate a little phloroglucinol was added; upon heating on a boiling water bath, a red color developed. This indicated the presence of either galactose, pentose or glycuronic acid. The rest of the hydrolyzed material was heated for two hours on a boiling water bath with 1 ml of concentrated nitric acid. Upon standing over night a water-white crystalline material was obtained which showed the crystalline form of mucic acid (2,3,4,5-tetrahydroxyhexanedioic acid) under the microscope, but unfortunately the amounts were not sufficient for a satisfactory melting point. It seems very likely, however, that we were dealing with galactose, since neither pentose nor glycuronic acid would yield a precipitate upon heating with nitric acid.

The question whether the saliva-resistant fraction is galactogen or a galactose-containing polysaccharide *sui generis* cannot be answered definitely at the present time. The lowered value of the optical rotation of the original polysaccharide mixture as against pure glycogen would well agree with the presence of galactogen which shows in pure state an optical rotation of $\alpha \frac{20}{D} = -22.7^\circ$ (May, 1932). This latter polysaccharide has been found so far in various molluscs and in vertebrates (May, 1934). Its presence in a representative of a third phylum would not be surprising. Basing the calculation on the available figures for optical rotation, it might be assumed that the original polysaccharide mixture contained about 4% galactogen, corresponding roughly to 0.04% of the fresh weight of the worms. For a complete satisfactory analysis therefore about 10 kg or about 2000 adult females would be required, an amount difficult to collect.

INORGANIC SUBSTANCES

In view of the growing realization of the importance of the "trace elements" in many biological fields, it was decided to subject the worms to a spectrographic analysis. This seemed the more important as very

little is known about the inorganic constituents of any parasite. The most complete analyses are those of Flury (1912) on ascarids and those of Salisbury and Anderson (1939) on *Cysticercus fasciolaris*. The former found the metals Na, K, Ca, Mg, Fe and Al, whereas he was unable to demonstrate the presence of Cu and Mn. The latter mention as metals Na, K, Ca, Mg and Fe. In *Macracanthorhynchus* the presence of only the following metals has been shown in a qualitative way: Na, K, Ca, Mg and Fe (von Brand, 1939).

The worms intended for the spectrographic analysis were collected especially for this purpose, care being taken to avoid any contamination with metals (except, of course, Na, since the worms had to be washed in isotonic NaCl solution after isolation). They were dried, ground in a mortar and analyzed qualitatively with a quartz Littrow spectrograph.

Four different samples were analyzed: females; males; eggs and ovaries; body fluid of females after centrifuging off the eggs and ovaries. The following metals were found in all four samples: Na, Ca, Mg, Mn, Al, Fe and Cu. The range of wave length in which the characteristic lines of the elements were located was in the near ultra-violet. In this range potassium lines are very faint and their presence was not demonstrated. However, potassium lines appeared clearly in the far ultra-violet. In addition to the metals, the spectrograph revealed the presence of P and Si.

In view of the importance that Fe and Cu play in the constitution of respiratory pigments occurring in invertebrates (haemoglobin, haemocyanine), quantitative determinations of these substances were performed; although nothing is known at the present time about the presence or absence of any respiratory pigment in acanthocephalids. The sample used for these determinations consisted of dried females, containing 3.80% ash.

Iron was determined according to the method of Linzel (1933).

2.455 mg gave 7.2×10^{-3} mg Fe
2.775 mg gave 8.4×10^{-3} mg Fe
Found: Fe = 0.29, 0.30%

Copper was determined according to the method of Eisler, Rosdahl and Theorell (1936).

2.133 mg gave 0.000 mg Cu
9.998 mg gave 0.000 mg Cu

With this method Cu cannot be demonstrated if present in amounts less than 0.01%. Since all the spectrographic examinations were positive for Cu, it is legitimate to assume that its concentration was less than 0.01%.

SUMMARY

1. *Macracanthorhynchus hirudinaceus* contains besides true glycogen small amounts of a polysaccharide resembling and perhaps identical with galactogen.
2. Spectrographic analysis revealed the presence of the following metals in the worms: Na, K, Ca, Mg, Mn, Al, Fe and Cu.

BIBLIOGRAPHY

- BRAND, TH. VON 1939 Chemical and morphological observations upon the composition of *Macracanthorhynchus hirudinaceus* (Acanthocephala). J. Parasitol. 25: 329-342.
- 1940 Further observations upon the composition of Acanthocephala. J. Parasitol. 26: 301-307.
- EISLER, B., K. G. ROSDAHL AND H. THEORELL 1936 Ueber die Mikrobestimmung des Kupfers mit Hilfe der lichtelektrischen Photometrie. Biochem. Z. 285: 76-77.
- FLURY, F. 1912 Zur Chemie und Toxikologie der Ascariden. Arch. Exper. Path. u. Pharm. 67: 275-392.
- GATIN-GRUZEWSKA, Z. 1904 Das reine Glykogen. Pflueger's Arch. 102: 569-591.
- LINTZEL, W. 1933 Zur Methodik der Mikrobestimmung des Eisens in biologischem Material. Z. Exper. Med. 86: 269-274.
- MAY, F. 1932 Ueber den Galaktogengehalt der Eier von *Helix pomatia*. Z. Biol. 92: 325-330.
- 1934 Chemische und biologische Untersuchungen ueber Galaktogen. Biologischer Teil: Galaktogen Nachweis bei verschiedenen Tierklassen und beim Menschen. Z. Biol. 95: 614-634.
- SALISBURY, F. L. AND R. J. ANDERSON 1939 Concerning the chemical composition of *Cysticercus fasciolaris*. J. Biol. Chem. 129: 505-517.

CERTAIN NUTRITIONAL REQUIREMENTS OF THE FOWL
CESTODE *RAILLIETINA CESTICILLUS* (MOLIN)
AS DEMONSTRATED BY SHORT PERIODS
OF STARVATION OF THE HOST^{1,2}

W. M. REID

Kansas State College of Agriculture and Applied Science, and Monmouth College

Because of the lack of a digestive system in cestodes, the nutrition and the nutritional habits of tapeworms have long interested parasitologists. However, due primarily to the difficulties involved in studying the processes which normally occur only within the intestine of the host, comparatively little is known about the food requirements of this unique group. All attempts to simulate normal conditions of warm-blooded animals by the use of in vitro methods have met with disappointment as mere survival of tapeworms for not more than a few days is all that has thus far been accomplished. Nevertheless, most of the knowledge of the physiological processes of tapeworms has come from the in vitro experiments of such workers as von Brand (1933) and Wardle (1937a). In this type of work portions of *Moniezia expansa* were subjected to various treatments for a few hours, analyzed chemically, and their composition compared with that of untreated portions of the worm which had been previously analyzed.

Another type of experimental study has been made by Levine (1938) and more recently by Hager (1941) in which the diet of the host was altered in various ways and the effects upon proglottid production or onchosphere count were measured. It appears that only von Brand has combined the two methods by a study of chemical composition of worms after the host has been starved or given special diets.

Recent observations upon short starvation periods of chickens infected with *Raillietina cesticillus* (Molin) led to the conclusion that rather profound changes must be induced in these parasites by lack of proper food in the gut of the host (Reid, 1940). Therefore, a more comprehensive study was made by using the combination of starvation and chemical analyses of worms removed from starved and from control hosts.

MATERIAL AND METHODS

Both chickens and beetles (intermediate hosts used in producing experimental infections of the fowls) were reared according to the technique

Received for publication, October 27, 1941.

¹Contribution No. 229 from the Department of Zoology, Kansas Agricultural Experiment Station, Manhattan, Kansas, and the Department of Biology, Monmouth College, Monmouth, Illinois.

²A portion of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy. The assistance of Dr. James E. Ackert under whose direction the work was carried out is gratefully acknowledged.

of Reid, Ackert, and Case (1938) with certain modifications and improvements (Reid, 1942). Worms to be analyzed were removed immediately after the fowl had been killed by deeply scraping the mucosa in the region under the scolex. In many cases this served to free the deeply embedded scolex, but because speed was essential, and the weight involved negligible, the recovery of the scolex was not considered essential. The worms were quickly washed in mammalian Ringer's solution to remove any debris or mucus adhering to them and rolled on a dry piece of filter paper to remove surface moisture.

Worms to be analyzed for glycogen were placed immediately in 0.5 cc of 30 per cent KOH and the glycogen determinations made by the micro-method of Heatley (1935). Acid hydrolysis of the glycogen was carried out with a 2 N solution of sulfuric acid instead of the usual 2.2 per cent hydrochloric acid since Sahyun (1931) has shown that the chlorine ion interferes with the development of the final blue color when the Folin and Wu macro sugar method (1920) is used, while the sulfate ion does not so interfere.³

Worms to be used in fat determinations were removed in a similar manner, but larger numbers of worms were included in each sample. Immediately upon removal, the wet weight of the worms was obtained by weighing in small test tubes. The worms and the water that had oozed out in the weighing tube were placed on weighed, ash-free, fat-free filter paper and permitted to dry at room temperature. Last traces of moisture were removed in vacuo over calcium chloride. The ether-soluble portion was extracted by means of a Bailey-Walker fat extraction assembly. After weighing back the filter paper containing the residual material, Kjeldahl determinations for organic nitrogen were made on some of the samples while other samples were ashed in a muffle and the ash content determined.

REVIEW OF LITERATURE

NUTRITION

Because of the economic importance of the nematodes, the nutritional habits of this group have been more extensively studied than those of other helminths. Lapage (1937) in his comprehensive review of the nematodes included a section on nutrition and its effects on the host. Ackert and Whitlock (1941) have reviewed the feeding habits of the nematodes. McCoy (1935) reviewed the physiology of the helminth

³ Although it is customary to multiply the reducing sugar value by Pflüger's correction factor of 0.927 to obtain true glycogen values, this figure has recently been questioned. Kerly (1930) used 0.957 while Wardle (1937a), calculating from results obtained by Bell and Young, used 0.961. Following the example of some biochemists in waiting for the question to be settled, no correction of the reducing sugar values has been made in the present studies.

parasites including a section on nutrition. Histological examination of the gut and its contents as well as numerous ingenious experimental procedures have shown that there is wide variation in the food and feeding habits of the nematodes. These differences are generally correlated with the position of the parasite within the host. Some are saprozoic, living on digested food of the host; others live on bacteria; while others feed on tissue, tissue exudate or body fluids. Although equipped with a digestive system, some forms such as the filaria probably absorb nutriment through the body wall.

Studies on fluke nutrition are less extensive, but there appear to be similarities with the modes of nutrition of the nematodes. Hsü (1938) in a study of both nematode and trematode tissue-parasites found a higher correlation between the location of the parasite and its food than between food and the phylum to which the parasite belonged. Thus *Paragonimus ringeri* and *Spirocerca sanguinolenta* which live in nodules, feed on cells passing through the wall of the nodule, while *Schistosoma japonicum*, *Dirofilaria immitis*, and *Rhabdias* sp. which live in the vascular system, take only cells present in the general circulation.

Entirely different methods must be applied to the study of the nutrition of cestodes since no digestive system is present; the food must therefore consist of easily diffusible substances present in the gut of the host. Most of the evidence available on the nutrition of this group has come from physiological studies, a review of which was made by Wardle (1937b).

It is now well established that cestodes, as well as other intestinal helminths, contain large quantities of glycogen and fat. According to von Brand (1933), glycogen plays the major rôle in the metabolism of this group, while fat is at least partially a waste product. He recorded slight decreases in glycogen content in sections of *Moniezia expansa* after six hours of in vitro starvation. In similar experiments in which glucose was added to the media, no significant change in the glycogen content was noted.

Wardle (1937a) pointed out that although the polysaccharide content of the worms is generally conceded to be synthesized from glucose, there is little experimental evidence to support this view. Wardle's experiments, likewise with *M. expansa* during six hours of in vitro starvation, suggest that glycogen is consumed in quintuple Tyrode, one per cent sodium glycocholate solution, and in bile saline. He pointed out that although these results are suggestive of polysaccharide loss due to increased function of the musculature in these solutions, the possibility of glycogen loss by exosmosis must be considered. These results would be more convincing were sufficient chemical analyses available so that statistical methods could be used as the criteria of significance, in place of

the extreme range in variation of four controls. By similar methods Wardle reported gains in polysaccharide content when other sugars were substituted for glucose.

More recently Markov (1939), who has been able to maintain worms under in vitro conditions for longer periods of time, has demonstrated that glucose is absorbed by the cestodes *Ligula intestinalis*, *Eubothrium rugosum* and *Diphyllobothrium latum*, and the nematode *Triaenophorus nodulosus*, and that the rate of glycogen consumption is independent of the initial glycogen content.

Another type of evidence for the need of carbohydrate food in cestodes was presented by Levine (1938). Indigestible substances such as bran or cellophane, when added to the host diet in sufficient quantity to provide the necessary bulk but also to greatly reduce the carbohydrate content, caused a marked reduction in proglottid production in *Davainea proglottina*.

Experiments with other factors in the diet of the host upon cestodes have been conducted by Hager (1941) and Alicata (1939, 1940). Hager showed that a factor, associated with the vitamin G complex, was necessary for normal oncosphere production by the rat tapeworm *Hymenolepis diminuta*. Alicata found indications that a diet high in animal-protein permitted fewer worms to develop than a diet high in plant-protein in experiments with *Hymenolepis exigua* of the fowl. The dietary effects produced in this latter experiment confirm similar findings with the nematode *Ascaridia lineata* by Ackert and Beach (1933).

STARVATION

The effects of starvation of the host upon the intestinal fauna seem to have had little systematic study, but the literature contains several interesting observations on the subject and starvation has been used as an experimental tool in isolated cases.

In the trematodes Beaver⁴ observed that "for *Echinostomum revolutum* the general well being of the host, including nutrition, directly influenced the parasite. Sick or starved hosts lost their worms." Burlingame and Chandler (1941) noted that fasting rats lost the acanthocephalan worms *Moniliformis dubius*, 65 out of an estimated 227 worms in 12 rats having been lost in five days.

In studies on the food of the fowl nematode *Ascaridia lineata*, Ackert, Whitlock and Freeman (1940) showed that worms in chickens given only water per os grew very little during the first eight days of starvation and not at all between eight and 27 days. Although both control and experimental chickens lost worms during the course of the starvation period, the experimental chickens lost them at a much faster rate than did the controls.

⁴ Personal correspondence, April 21, 1941.

With the cestode *Davainca proglottina*, Levine (1938) found that complete starvation of the host for 24 hours caused a decrease in proglottid production during the following week.

In conjunction with the present work, a detailed study (Reid, 1940, 1942) of the effects of starvation on *Railletina cesticillus* revealed that starvation of hosts infected with this worm for 24 to 48 hours resulted in the loss of the strobila of all of the worms present in the gut of the host. The scolex, however, was unaffected by starvation periods which extended as long as 20 days, and was able to regenerate a new strobila upon resumption of normal feeding habits in the host. Furthermore, newly established cysticeroids, although sometimes able to maintain their respective positions in an empty gut, did not grow appreciably until the fowl was placed upon normal feed.

EXPERIMENTAL DATA

CARBOHYDRATE CONTENT OF WORMS BEFORE AND AFTER SHORT PERIODS OF HOST STARVATION

Chemical determination of possible food reserves in conjunction with host starvation were undertaken to determine whether this loss of strobilae in starved hosts was due to an actual starvation of the worm tissue or to some other factor in the host-parasite relationship. Since it has been established that glycogen is the predominant carbohydrate in tapeworms as well as other intestinal parasites and since it is likewise well-known that the glycogen store of higher animals is readily depleted dur-

TABLE 1.—Glycogen level in tapeworms from a starved and an unstarved host. (Exper. 1)

Hour killed and treatment	Chicken number	Total worms	Worms in sample	Per cent glycogen of worm wet weight
4 PM On feed until killed	A 897	10	1	4.83
			1	3.62
			1	2.52
			1	4.38
			1	4.55
			1	6.45
			1	4.85
			1	5.25
			1	4.95
			Mean	4.60
6 PM Off feed for 24 hours before killing	A 1092	17	1	0.09
			1	0.06
			1	0.10
			1	0.03
			1	0.32
			1	0.49
			1	0.47
			3	0.46
			3	0.18
			Mean	0.25
Difference in means			4.35*	
Standard error			0.387	

* Highly significant.

ing starvation, it followed that glycogen might be the logical food reserve to investigate first.

Exper. 1.—The first glycogen analyses were made upon individual worms from a chicken killed at 4 PM after it had been feeding normally, and upon worms from a chicken which had been off feed for 24 hours. The mean glycogen percentage calculated on the basis of the wet weight of the worms for nine cestodes from the unstarved chicken was 4.60 per cent (Table 1) while that on nine analyses of worms from starved hosts was 0.25 per cent. Although the glycogen level of the latter group of worms was so low that the glucose readings had to be estimated, in all cases these estimates were probably high. It is apparent, therefore, that the glycogen content of the worms in the starved host was reduced to a mere trace. Furthermore, it was of interest to note that the worms removed from this chicken were flaccid and very much less active than worms from the normally fed chicken.

Exper. 2.—While 23 tapeworms were available for study in *Exper. 1*, these worms were from but two chickens. Accordingly, a second experiment was planned in which worms from eight chickens were analyzed, four of the chickens being normally fed, and four having been without feed for 20 hours before the worms were removed. The starvation time in this experiment had to be reduced by four hours since repeated efforts again to obtain whole worms from chickens starved for 24 hours met with failure. Another change in procedure was the use of more worms per sample so that sufficient glucose was present for accurate determination. It may be noted (Table 2) that worms from the control chickens had a mean glycogen level, calculated on a per chicken basis, of 4.38 per cent with a range per chicken of 3.98 to 5.14 per cent. With

TABLE 2.—Glycogen level in tapeworms from several starved and unstarved chickens. (*Exper. 2*)

Hour killed and treatment	Chicken number	Total worms per chicken	Number of worms per sample	Per cent glycogen of worm wet weight	Mean per cent glycogen in worms
6-7 PM On feed until killed	A 1095	44	10	3.84	4.34
	A 1015	36	10	4.83	
			7	4.67	
	A 1363	3	7	5.60	
	A 1096	107	2	3.98	
			30 scoleces	4.08	4.08
				Mean	4.38
2-3 PM Off feed 20 hours before killing	A 1100	56	10	0.24	0.38
			10	0.51	
	A 1094	33	10	0.25	
			10	0.42	0.34
	A 1098	33	10	0.57	0.57
	A 1097	200	30	0.21	0.19
			30 scoleces	0.17	
				Mean	0.37
				Difference in means	4.01*
				Standard error	0.274

* Highly significant.

the starved chickens the mean per bird level was 0.37 per cent with a range between 0.19 and 0.57 per cent. The mean difference of 4.01 per cent between the two groups is highly significant.

The results of Exper. 1 and 2 show clearly that this marked fall in glycogen level is due to host starvation. Furthermore, it seems probable that this drop in glycogen content of worms in starved hosts accounts for the loss of strobilae from these worms. Ortner-Schönbach (1913) in a histological study of the distribution of glycogen in cestodes reported concentrated clumps of glycogen in the parenchyma around muscle fibers, the muscle fibers themselves being relatively free of glycogen. She presumed this glycogen to be a source of muscular energy. In the present experiments it would follow that when the glycogen supply around these muscle fibers is exhausted the muscles would no longer be able to contract normally. Using an analogy introduced by Wardle (1935), the worm is no longer able to swim effectively against the current in the intestine and is swept out by peristaltic action. The flaccid condition of worms from starved hosts would tend to support this view. Since the scolex of this species is very deeply embedded and the neck region is relatively fragile, separation usually occurs at this point.

GLYCOGEN FLUCTUATIONS DURING THE NORMAL TWENTY-FOUR HOUR CYCLE

If such a profound change in glycogen level in the worms could occur during 20 hours of host starvation which includes 12 hours of night, it seemed likely that fluctuations in glycogen content might occur during the normal 24-hour feeding cycle. Such a fluctuation would be of special interest because of the relationship between food and the proglottid production cycle. Wetzel (1932) reported such a cycle for the fowl cestode *Davainea proglottina*, a peak in proglottid production occurring at about 3:30 PM with a gradual decrease to complete cessation during the early morning hours. In 1934, Wetzel reported similar cycles for *R. cesticillus*. The occurrence of these cycles has been confirmed by Reid, Ackert and Case (1938) for *R. cesticillus*. Levine (1938) has likewise confirmed the presence of these cycles in *Davainea proglottina*. He was able to reverse the peak to the early morning hours by changing the feeding time to the night. He thus effectively demonstrated a fact that had been suspected by other workers, namely; that this proglottid production cycle was dependent on the feeding habits of the fowl.

Exper. 3.—In order to test the possibility of a fluctuation in the glycogen level in the 24-hour cycle, worms from two groups of chickens were analyzed (Table 3). One group was killed between 6 and 7 PM, which was at the close of the normal feeding period in November, while the other group was killed between 6 and 7 AM, just before feeding had

begun. The glycogen level when calculated on a per worm basis (only samples consisting of one worm each were included in the statistical analysis) was 7.14 per cent at the evening hour while it averaged only 3.68 per cent at the early morning hour. The 12 worms analyzed from the group killed during the evening thus had an average of 3.46 per cent

TABLE 3.—Glycogen level at the beginning and at the end of a normal feeding period. (Exper. 3)

Hour killed and treatment	Chicken number	Total worms	Worms in sample	Location in gut cm from ampulla	Per cent of worm wet weight	Mean per cent glycogen in worms
6-7 P.M. On feed until killed	9706	2	1	8	5.72	5.94
	9703	2	1	14	6.17	
	9716	4	1	10	8.51	8.23
			1	10	7.85	
			1	8	8.21	
			1	10	7.75	
	9709	35	1	13	8.20	8.11
			1	13	8.29	
			1	2-8	5.31	
			1	12	6.18	
			1	13-18	6.87	
			1	18-23	6.49	
			2	30-35	6.44	
			2	41	6.34	
			Means		7.14	
						6.30
						7.13
6-7 A.M. Off feed during night	9724	33	1	8-10	4.30	4.37
			1	8-10	3.81	
			1	8-10	3.54	
			1	8-10	3.66	
			1	10-16	4.69	
			1	16-20	4.38	
			1	20-25	4.41	
			1	20-25	5.38	
			1	20-25	5.15	
			1	30-38	5.06	
			2	30-38	4.36	
			19	random	4.33	
	A 1122 9711	3 9	1	15	3.34	3.34
			1	8	2.14	
			1	10	3.64	
			1	18	3.68	
			1	35	3.43	
			1	35	3.77	
			1	40	3.60	3.34
			1	45	3.01	
			1	45	3.44	
			1	45	3.44	
			Means		3.68	3.92
			Difference in means		3.46*	3.21*
			Standard error		0.763	0.346

* Highly significant.

more glycogen than 19 worms in the morning group. As the standard error was 0.763, the difference was highly significant (Table 3). Likewise, when the glycogen level was calculated on a per chicken basis, the four-chicken group killed between 6 and 7 P.M. averaged 7.13 per cent while the three chickens in the 6 to 7 A.M. group averaged 3.92 per cent. The difference of 3.21 per cent in this case was likewise highly significant. It has been found, therefore, that upon whatever basis comparison is made, the glycogen level during the evening hours was markedly higher than that in the early morning hours.

These data are convincing evidence that the glycogen level fluctuates

during a normal 24-hour feeding cycle. It is higher in the evening after feeding has been in progress during the day, but lower during the early morning hours when no food is in the environment of the tapeworm. It seems probable that this rhythmic cycle in glycogen level during the 24-hour period is responsible for the cycle in proglottid production of the tapeworm. No explanation of this proglottid production cycle has thus far been suggested other than the correlation between it and the feeding habits of the host.

In weighing worms from different locations in the intestine for glycogen analyses, it was noted that the worms at the anterior end of the gut weighed appreciably more than those in posterior locations. In some cases those in anterior locations, although no longer than those in posterior positions, weighed twice as much. This raised the question as to whether a difference in the glycogen content due to different food conditions in the gut might be responsible for this difference in size. Study of the results indicated in Table 3, however, does not reveal any significant trends of variation in the glycogen content of worms from different parts of the gut.

Exper. 4:—To further correlate the glycogen cycle with the proglottid production cycle, analyses were undertaken at the mid-morning hour. Worms were extracted at 10 AM from four chickens which had been on feed and from five chickens which had been off feed during the morning of the experiment. The glycogen content was calculated on a per chicken basis as determined from either two or three samples consisting of two worms each. The percentages were 1.08, 1.48, 2.08, 3.00, for the chickens with feed before them continuously, while the chickens which had been off feed for 20 hours contained worms with 2.08, 1.57, 1.73, 1.90 and 1.23 per cent glycogen. Since no significant difference in the glycogen levels in these two groups was demonstrated, it must be concluded that the expected rise in glycogen content after the relatively low level of the early morning hours had not begun to take place. There is, apparently, a time lag in the building up of the glycogen store since the control birds had all begun to feed three hours before they were killed.

The data from these four experiments together with some additional data are summarized in Fig. 1. It should be noted that the first two-thirds of the curve represents the normal glycogen cycle of the cestode which is repeated from day to day, while the last third represents the glycogen levels when feed is removed the following day. The perpendicular bars represent twice the standard error.

Uncontrolled fluctuations in feeding habits of the birds, due to shortening daylight hours while the experiments were in progress, and the small number of points at which determinations could be made, make it impossible to draw conclusions from minor trends in the curve. How-

ever, the three major trends previously mentioned are emphasized, namely: (1) the sharp decrease in glycogen content to a mere trace when food is withheld from the host, (2) a fluctuating daily cycle with a peak in the evening and a low point during the mid-morning and (3) a time lag between the beginning of feeding and the building up of a glycogen store. Comparison of curves in the glycogen cycle in the present work with those in the proglottid production cycle of Wetzel show marked similarity. From this it may be inferred that the latter cycle is dependent upon the former.

Exper. 5.—Although only entire worms which averaged 22.9 milligrams from starved chickens were used for analysis, the worms extracted

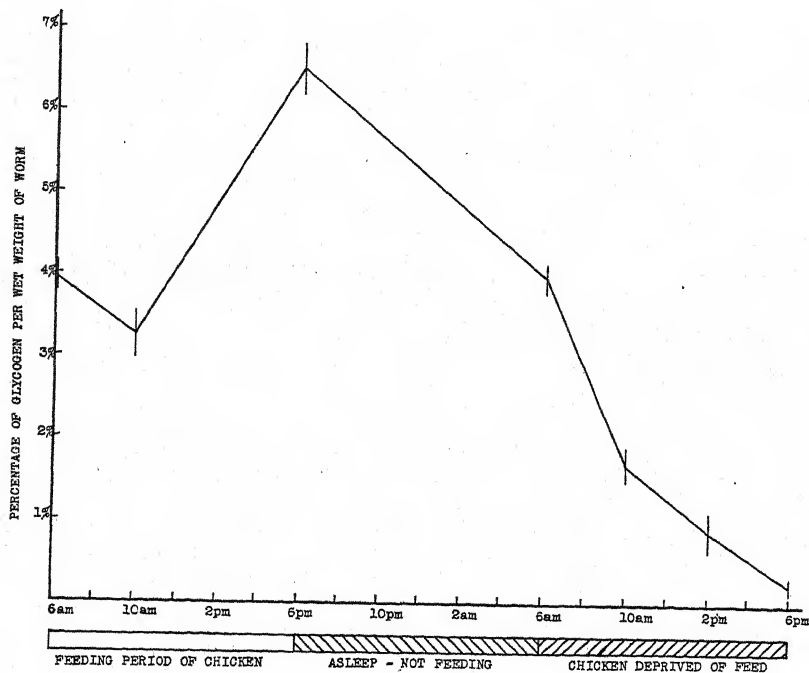


FIG. 1. Fluctuations in the glycogen level of tapeworms from chickens feeding normally, and during short periods of forced starvation.

were on the average a few milligrams lighter than those from unstarved chickens. Since this difference in weight was undoubtedly due largely to the loss of terminal proglottids in the worms from starved chickens, the question might be raised as to whether or not a glycogen gradient existed within each individual worm. Should the gravid proglottids which were lost prove to have a considerably higher glycogen level than that of the rest of the worm, the explanation given to the foregoing glycogen experiments would need to be reinterpreted.

Accordingly, an experiment was devised in which the 20 posterior segments from each worm were removed and separate analyses made on the posterior and the anterior portions of the worms. In small worms the 20 proglottids constituted about half the total weight of the worm, while in larger worms they made up a much smaller portion of the worm weight. Worms were obtained from chickens killed at 8 PM and 8 AM, so that further comparisons in the glycogen level between the evening and morning hours could be made.

The mean percentage of glycogen for the anterior ends of eight samples removed from three chickens at 8 PM was 6.54 while that of the posterior ends of these worms averaged 5.55 per cent. There was a difference of 0.99 per cent in the glycogen level in favor of the anterior ends, and the standard error being 0.295, the difference is highly significant. At the 8 AM hour the mean glycogen level for nine samples taken from four chickens was 5.01 per cent for the anterior ends while that of the posterior ends was 5.15 per cent. In this case the difference was not significant.

The results of these two groups of tests show that the glycogen level from the posterior proglottids was not significantly higher than that of the anterior portions. Although further experimentation would probably be necessary to indicate the reason for the higher glycogen level in the anterior ends at the 8 PM hour, it appears improbable that a glycogen gradient could prejudice the conclusions drawn from the previous experiments.

As might be expected from the results of Experiment 3, the glycogen level at the 8 PM hour was higher than at the 8 AM hour, the 0.96 per cent difference in the means with a standard error of 0.250 is highly significant. Unfortunately the fowls, which were not accustomed to the operation of a new battery feeder, did not feed normally the day before the worms were removed, and the glycogen levels obtained in this experiment were not comparable with those indicated in Fig. 1.

PERCENTAGE COMPOSITION OF *R. CESTICILLUS*

Since reports of the percentage composition of other cestodes are available only in rather inaccessible sources, these data are presented in Table 4, where ready comparison can be made with the results obtained in the present experiments. It will be noted that the glycogen level for *R. cesticillus* with a mean of 6.51 per cent at the 6 PM hour is higher than the results obtained by other investigators with the exception of the 7.37 per cent recorded by Smorodinezew and Bebeschin (1935) for *T. saginata*.

FAT CONTENT AND STARVATION

Considerable interest has been shown in the fat metabolism of hel-

TABLE 4.—Percentage composition of the strobilar phase of cestodes analyzed by various investigators expressed as the mean with the range given in parentheses

Cestode	Investigator	Water %	Glycogen %	Ether-soluble extract %	Total nitrogen %	Ash %
<i>R. cesticillus</i>	Reld	79.46 (75.5-83.1)	6.51 (3.84-8.51)	3.20 (1.35-4.02)	1.14 (1.04-1.41)	2.36 (1.86-3.05)
<i>M. expansa</i>	Wardle 1937a and 1937b	85-90	3.22*			1-2
"	Weinland 1901	90.1 (89.5-92.2)	3.00† (1.55-5.96)*			
"	v. Brand 1933	88.96 (86.79-91.51)	2.69† (1.5-4.7)†			
<i>D. caninum</i>	"	79.61	(0.99-5.35)	3.35 (1.91-4.56)	0.63 (0.56-0.76)	1.11 (1.02-1.20)
<i>T. marginata</i>	"	76.44 (78.32-74.56)				
<i>T. plicata</i>	"	72.50		9.11		1.22
<i>M. denticulata</i>	"	91.42				
<i>T. saginata</i>	"	92.23				
"	Smorodinezew, Bebe- schin, and Pawlowa, 1933, 1935, and 1936	87.82 (86.8-88.6)	7.37† (6.16-9.12)†	1.36 (0.20-3.17)	0.64 (0.54-0.72)	0.65 (0.52-0.85)
<i>T. solium</i>	"	91.29 (87.27-94.08)	2.17† (1.00-6.90)†	1.41 (0.83-2.77)	0.65 (0.47-0.93)	0.53 (0.29-1.03)
<i>D. latum</i>	"	90.50	1.90† (0.80-4.64)†	1.54	0.91	0.43

* Correction factor of 0.961.

† Probable correction factor 0.927.

minths because of the relatively high fat content, and the wide variability found in worms of the same species (Table 4). According to von Brand's view fats are byproducts of glycogen metabolism which probably remain as stored excretory material in the parenchyma of the worm. This conclusion was reached when relatively little fat was found in the excretory tubules of *M. expansa* as compared with large numbers of fat droplets found in the excretory sytem of the young fluke, *Fasciola hepatica* (Vogel and von Brand, 1933). Von Brand, however, found no significant change in fat content in nine experiments using in vitro starvation for six hours.

The results from ether extraction of 20 samples of *R. cesticillus* are summarized in Table 5. It will be noted that this species also has a

TABLE 5.—Ether-extract and percentage of water in worms removed from chickens off feed for 16 to 20 hours and upon worms from unstarved chickens

Treatment of worms	Total worms in sample	Water %	Ether-extract of wet weight %	Ether-extract of dry weight %
Unstarved	41	83.1	1.55	9.2
Chickens killed between 2 PM and 6 PM	14	77.5	2.78	12.2
	31	77.6	3.69	16.5
	73	82.6	4.48	25.8
	93	80.1	3.76	19.0
	11	76.3	4.62	19.4
	24	78.4	2.38	10.9
	11	75.5	4.05	16.6
	100	80.7	3.30	17.1
	26	82.8	1.35	7.9
	Means	79.46	3.20	15.5
Starved	23	78.6	3.38	15.7
16-20 hours	48	83.4	3.24	19.6
Chickens killed between 2 PM and 6 PM	31	83.6	3.10	18.9
	38	83.5	3.08	18.4
	22	80.7	4.41	22.8
	39	85.7	1.62	13.0
	38	84.5	1.36	7.5
	25	81.2	1.90	9.9
	22	82.0	2.86	15.8
	45	86.5	2.00	15.0
	Means	82.97	2.70	15.5
Difference in means		3.51*	0.50	0.0
Standard error		1.226	0.466	...

* Statistically significant.

relatively high ether-soluble portion, the mean for the control samples being 3.20 per cent of the wet weight. In nine additional samples from worms killed in the morning hours, and therefore not included in the starvation table, the mean was 3.31 per cent with a range of 0.98-4.51 per cent.

Although the worms from starved chickens contained an average of 0.50 per cent less ether-soluble material than the control samples, the difference is not statistically significant. Of interest is the fact that the means are the same for the ether-soluble portion calculated on the basis of dry weight. Since a loss of approximately 30 per cent in the dry

weight of the worm would occur if all of the metabolic products of glycogen consumption during starvation were eliminated, a corresponding gain in the ether-soluble fraction might be expected if no actual change in its content had occurred. The present results might, therefore, be interpreted as indirect evidence for a decrease in the fat content during starvation.

The indications are that if an excess of fatty waste products was produced during starvation, due to the high glycogen consumption, these substances must have been excreted by the worm and did not accumulate in the parenchyma. On the other hand, the evidence is inconclusive for the opposite view that fats are a potential energy reserve, since the depletion is slight if a true loss does actually occur.

WATER CONTENT AND STARVATION

The water content of some thirty specimens was determined in connection with fat determinations (Table 5). It may be noted that the water content of the worms from starved chickens was 3.51 per cent higher than that from unstarved hosts. The standard error being 1.226, this difference is significant. Since there was a marked depletion in the glycogen content of the worm during starvation, a corresponding rise in water content might be expected. It should be noted, however, that starved hosts had water before them continually and therefore an increase in water content in the worms might be due to the increased percentage of water in their surroundings. The water content of this species, although not the lowest of the worms which have been analyzed, is lower than any of the other forms upon which complete determinations have been made. This is reflected in the higher percentage of glycogen, ash, and nitrogen reported for this species.

PROTEIN CONTENT AND STARVATION

Nitrogen determinations have been made on cestodes by several investigators to obtain an approximation of the protein present. The usual procedure is to multiply the total nitrogen by 6.25 to obtain the percentage of protein. Although no complete study of the protein content of cestodes has been made, Salisbury and Anderson (1939) in an analysis of the larval *Cysticercus fasciolaris* found that of 20 per cent of the total nitrogen which was dissolved in aqueous extracts of pulverized worm material, only seven per cent was precipitated by trichloroacetic acid and could therefore be regarded as protein. Likewise Eisenbrandt (1938) in a serological study of numerous helminths including the cestodes *Cittotaenia denticulata*, *Dipylidium caninum*, *Taenia pisiformis*, *Moniezia alba*, *M. expansa*, and *Thysanosoma actinioides* found the non-protein fraction of the total nitrogen to be surprisingly high. Although

it would thus appear that the conversion factor will probably have to be revised when a more complete knowledge of the protein make-up of tapeworms has been obtained, the nitrogen content still remains the most convenient method of obtaining an estimate of protein content.

Five nitrogen determinations were made upon worms extracted from unstarved hosts killed at 6 PM and six determinations were made on worms removed from hosts which had been starved for 16 to 20 hours. Calculated on the basis of the wet worm weight, the worms from unstarved hosts averaged 1.14 per cent nitrogen while those from starved hosts averaged 1.37 per cent. The difference of the means is 0.24 per cent with a standard error of 0.093 which is statistically significant. Calculated on the basis of dry weight, worms from the starved hosts showed even a greater increase, nitrogen constituting 7.87 per cent of the weight as against 5.83 per cent for the control group. The difference in this case is 2.04 per cent with a standard error of 0.277 which is highly significant.

In the light of the glycogen experiments it seems probable that the increase in nitrogen is relative rather than actual. Since there is at least no loss of nitrogenous materials, and little if any loss of fat during the 20-hour starvation period, it appears that the starvation effect under consideration is due entirely to loss of glycogen. These findings are in keeping with the prevailing opinion that glycogen furnishes the main source of energy for cestodes.

ASH CONTENT

The total ash content of nine samples of worms taken from unstarved hosts was determined. The ash content averaged 2.36 per cent of the wet weight of the worm material with a range between 3.95 and 1.96 per cent. Calculated on the basis of the dry weight, the mean percentage of ash was 12.11 with a range between 9.82 and 17.07 per cent.

Although the strobilar ash contents of adult cestodes have not been analyzed, Salisbury and Anderson (1939) determined the following elements in the ash of the larval *Cysticercus fasciolaris* which constituted 16.3 per cent of the dry material: Na 6.99 per cent, K 3.66 per cent, Ca 29.5 per cent, Mg 20.14 per cent, S 0.74 per cent, P 7 per cent, with only traces of Cl and Fe.

THE EFFECTS OF CROWDING

A phenomenon which appears to be common to the larger species of cestodes that have thus far been studied experimentally is that crowding causes stunting in the size of the worms. Woodland (1924), Shorb (1933), and Hunninen (1935), have demonstrated this effect in the rat tapeworm *Hymenolepis fraterna*. Hunninen stated further that even in heavy infections a few worms may grow materially larger than others,

while some of the worms may remain immature for long periods. Chandler (1939) and Hager (1941) likewise presented evidence on the effects of crowding upon the size of *Hymenolepis diminuta*.

In the present studies the weights of some 1270 specimens of *R. cesticillus* from 44 chickens were available for comparison. Since weight is the most accurate measure of size and a much more dependable criterion in cestodes than the length or number of proglottids, these data are here presented. The results are summarized in Fig. 2. It will be noted that

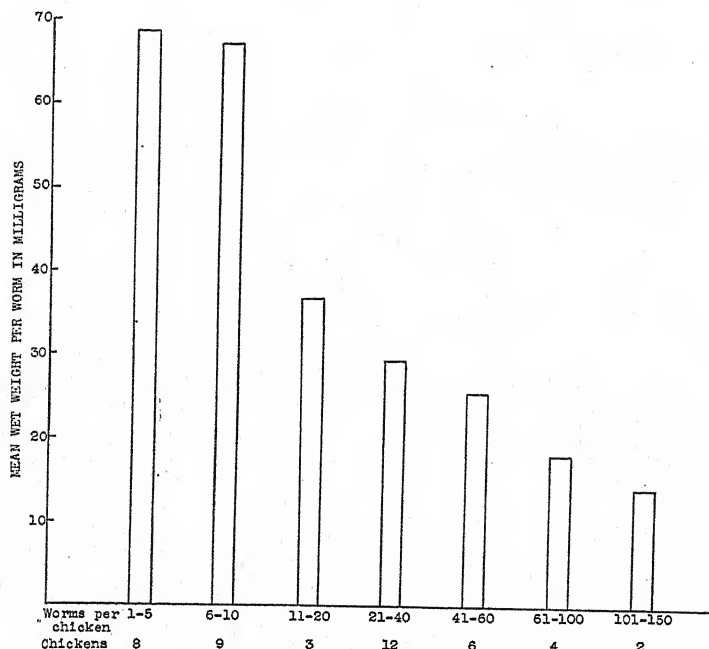


FIG. 2. The relative size of *R. cesticillus* in light and in heavy infections.

although little difference in size of worms is indicated between the one to five-worm group and the six to ten-worm group, a worm burden of over ten cestodes per bird results in a marked decrease in the size of the worms. Although the data available are insufficient to make numerical comparisons, it seems probable from observation that single worms reach greater sizes than do worms in two to ten-worm infections.

Four different causes have been suggested for this stunting effect in heavy infections. These are: (1) That a local immunity is developed in the host, (2) that insufficient food is available for all the tapeworms, (3) that excretory products of the worms inhibit growth, and (4) that an actual physical crowding takes place. There is some evidence against certain of these suggestions but no positive supporting evidence is available.

Chandler found that rats treated with an anthelmintic produced as large worms as similarly treated uninfected rats, and concluded that there is no evidence of a local immunity mechanism being involved in the dwarfing of tapeworms.

Even though it has been shown in the present studies that lack of sufficient carbohydrate in the gut of the chicken prevents the normal behavior of the tapeworms, the available evidence does not support the view that dwarfing in crowded infections is due to insufficient carbohydrate food. No significant difference in glycogen content of worms in heavy and in light infections could be found. Further support for the view that carbohydrate depletion is not responsible for dwarfing can be obtained indirectly by estimating the maximum amount of carbohydrate consumed by a heavy infection of worms. Judging from the data in Experiment 3, a large worm weighing 100 mg would scarcely be expected to consume more than 0.008 gm. of carbohydrate in a 24-hour period. Calculating from Henry's (1912) table, digestible carbohydrates would be present in the smallest amount of balanced ration consumed in a 24-hour period by any fowl under observation (16 gm), in excess of 8.5 gm. If 100 very large worms were present, not more than 10 per cent reduction in the sugar content of the gut would be expected. This would not appear to be sufficient to produce dwarfing. Hager (1941) showed experimentally that limiting the food of rats to one-half or one-third the normal intake had no effect on the reproductive rate of *H. diminuta*, reproduction likewise being inhibited by crowding.

WORM NUMBER AS DETERMINED BY PROGLOTTID COUNT

To compare the fowl hosts effectively, it became necessary to ascertain the approximate number of worms in a living chicken. In nematode studies such a procedure has been in use for many years, the worm number being determined by egg counts. Stoll (1936) by study of proglottid and onchosphere counts of the sheep cestode *Moniezia expansa* together with other biological data was able to determine single worm infections of this species. Levine (1938) was able to predict approximately the number of *Davainea proglottina* present in a fowl by proglottid count, each worm producing about one proglottid per day. Hager (1941) found no relationship between the onchosphere count and the number of *Hymenolepis diminuta* present in rats.

In estimating the number of *R. cesticillus* present in a fowl, two difficulties are encountered in making use of 24-hour proglottid counts. The most serious difficulty is related to the problem of crowding which has been discussed. From the results obtained in several hundred fecal examinations with various sizes of infections it may be concluded that in infections of one to ten worms, no crowding effect manifests itself in pro-

glottid production. In single worm infections or light infections, each worm produced an average of about nine proglottids per day. However, in heavy infections it was found that about four proglottids per day is a more usual number for each tapeworm. On this basis, low and high proglottid counts can be used with some accuracy. There is, however, an intermediate proglottid number by which determination cannot be made. The size of the proglottids may be of some value in making a determination under these conditions as gravid proglottids are larger in light than in heavy infections.

A second factor influencing the determination of worm numbers by proglottid counts is the duration of the infection. In the present studies occasional proglottids were recovered in the feces 11 days after the cysticercoids were fed. On the other hand, Jones is quoted by Stafseth (1935) as having noted the presence of the original terminal proglottid in certain worms a month after infection. A gradual increase in the number of proglottids recovered in the droppings of a fowl from the eleventh to about the thirty-fifth day after cysticercoids were fed was noted in the present work. It has been found necessary, therefore, to wait until about 30 days after infection to make a calculation by the proglottid count. A later gradual decline in the proglottid count is due to the loss of some worms, and to the more frequent periods of quiescence, in old infections.

The number of cysticercoids fed to a chicken may help in determining the number of tapeworms, but the percentage of infection is quite variable due to variations in hosts and in the viability of the cysticercoids. This knowledge, relative to cysticercoids ingested in combination with the proglottid count, may be helpful in estimation of the extent of infection. Estimation of worms present by recovering the original terminal proglottid from each worm has been found impractical. Although no simple formula can be applied to determine the number of worms in the host, the proglottid count combined with a knowledge of other biological factors makes possible a rough estimate of the minimum number of worms in a chicken.

DISCUSSION

The relatively high rate of glycogen consumption by the fowl tapeworm *R. cesticillus* revealed in the present study is somewhat surprising when the results are compared with in vitro studies which have been made on the sheep cestode *M. expansa*. The consumption of over 90 per cent of the total glycogen store in 20 hours of host starvation, as reported in Experiment 2, seems remarkable when compared with about 10 per cent of glycogen consumption during six hours with *M. expansa* as reported by von Brand (1933). It is questionable, however, as to how far the results obtained for the avian *R. cesticillus*, which does not usually

exceed 0.1 gram in weight, can be compared with the mammalian *M. expansa* which weighs several grams. The higher body temperature of the avian host would indicate a higher metabolic rate in *R. cesticillus*. Von Brand (1938) believed that the size of the parasite might determine the type of metabolism in a helminth. Nevertheless, in the light of the present studies, the importance of muscular contractions in overcoming the action of peristalsis, a factor mentioned by Wardle (1937a), might well be reemphasized.

Although the effects of variable secretion of digestive juices under starvation conditions could not be eliminated in the present experiments, the fact that glycogen loss occurred not only when the host was starved but also during the normal 24-hour feeding cycle, and the fact that immature cysticeroids do not develop until food is at hand (Reid, 1942), lends support to the view that the worms expelled after host starvation were actually starved.

Protozoologists have long recognized the effects of diet upon the parasite (Hegner, 1923, Becker, 1937, Becker and Morehouse, 1935, 1936, Becker and Derbyshire, 1937), but helminthologists have not given the matter much consideration. The facts established in the present studies, the evidence of a high carbohydrate requirement in cestodes presented by Levine (1938), and the experiments with vitamins by Hager (1941) constitute a growing body of evidence indicating that the food of cestodes is extremely important in maintaining normal conditions for proper growth and development. In studying the effects of deficient diets upon host resistance to *Hymenolepis fraterna*, Shorb (1933) noted a retardation in the growth of the parasite and some loss in worms which he attributed to the direct effect of the diet upon the parasite. Other studies on host resistance and deficient diets contain only incidental reference to the effects of these diets upon the parasite. In view of the present evidence it would appear that greater attention should be given to the effects of host diet upon the parasite, and that studies on diet and host resistance should be preceded with a knowledge of the nutritional requirements of the parasite involved.

SUMMARY

1. Twenty hours of host starvation is sufficient to reduce the glycogen content of the fowl cestode *Raillietina cesticillus* (Molin) to about one-eleventh of its normal level. This reduction in the glycogen store is believed to be responsible for the loss of strobilae in starvation because insufficient muscular energy is available to counteract the action of peristalsis.

2. A daily fluctuation occurred in the glycogen level of tapeworms in the gut of chickens under normal feeding habits from 7.14 per cent at 6 PM to 3.68 per cent at 6 AM. It is thought that this fluctuation,

which is dependent upon the presence of available food material in the host gut, explains the release of proglottids only at certain hours during the day. These findings afford proof of the importance of glycogen in the metabolism of cestodes.

3. The mean percentage composition of *R. cesticillus* removed from the fowl under normal feeding conditions at 6 PM was found to be 79.46 per cent water, 6.51 per cent glycogen, 3.20 per cent ether-soluble extract, 1.14 per cent nitrogen, and 2.36 per cent ash content. Compared with other work, the percentage of water is considerably lower than that in the other forms which have been completely analyzed, with correspondingly high glycogen, nitrogen, and ash values for this worm.

4. Crowding of *R. cesticillus* in heavy infections causes a dwarfing in the size the cestodes as measured by weight. The diminished carbohydrate level in the host intestine due to food consumed by the worm does not appear to be sufficient to produce this effect.

5. Proglottid counts supplemented with rather complete infection data, are of value in estimating the number of worms in a living fowl—information of value in anthelmintic and certain other experimental fields.

BIBLIOGRAPHY

- ACKERT, J. E. AND T. D. BEACH 1933 Resistance of chickens to the nematode, *Ascaridia lineata*, affected by dietary supplements. Tr. Am. Micr. Soc. 52: 51-58.
- ACKERT, J. E. AND J. H. WHITLOCK 1941 Feeding habits of nemas (Parasitica). In "An Introduction to Nematology." J. R. Christie, Ed. Babylon, N. Y. Section II, Part 2: 350-355.
- ACKERT, J. E., J. H. WHITLOCK AND A. E. FREEMAN, JR. 1940 The food of the fowl nematode, *Ascaridia lineata* (Schneider). J. Parasitol. 26: 17-32.
- ALICATA, JOSEPH E. 1939 Parasitology. Rep. Hawaii Agric. Exper. Sta. 66 pp.
- 1940 Poultry parasites. Rep. Hawaii Agric. Exper. Sta. Rep., pp. 65-66.
- BECKER, ELERY R. 1937 Dietary control in experimental coccidiosis. Science 86: 403-404.
- BECKER, ELERY R. AND NEAL F. MOREHOUSE 1935 Effect of skim milk, lactose, vinegar and iodine on the quantitative character of a coccidian infection. Proc. Soc. Exper. Biol. and Med. 32: 1030-1031.
- AND ——— 1936 The effect of diet on the coccidian infection of the rat. J. Parasitol. 22: 60-67.
- BECKER, ELERY R. AND RUSSEL C. DERBYSHIRE 1937 Biological assay of feeding stuffs in a basal ration for coccidium-growth-promoting substance. II. Barley, rye, wheat bran, wheat flour middlings, soybean meal. Iowa State Coll. J. Sc. 12: 211-215.
- BRAND, TH. VON 1933 Untersuchungen über den Stoffbestand einiger Cestoden und den Stoffwechsel von *Moniezia expansa*. Z. Vergleich. Physiol. 18: 562-596.
- 1938 The nature of the metabolic activities of intestinal helminths in their natural habitat: aerobiosis or anaerobiosis? Biodynamica 41: 1-13.
- BURLINGAME, PAUL L. AND ASA C. CHANDLER 1941 Host-parasite relations of *Moniliformis dubius* (Acanthocephala) in albino rats, and the environmental nature of resistance to single and superimposed infections with this parasite. Am. J. Hyg. 33D: 1-21.

- CHANDLER, ASA C. 1939 The effects of number and age of worms on development of primary and secondary infections with *Hymenolepis diminuta* in rats, and an investigation into the true nature of "premunition" in tapeworm infections. *Am. J. Hyg.* 29D: 105-114.
- EISENBRANDT, LESLIE L. 1938 On the serological relationship of some helminths. *Am. J. Hyg.* 27: 117-141.
- FOLIN, OTTO AND HSIEN WU 1920 A simplified and improved method for determination of sugar. *J. Biol. Chem.* 41: 367-374.
- HAGER, ANNE 1941 Effects of dietary modification of host rats on the tapeworm *Hymenolepis diminuta*. *Iowa State Coll. J. Sc.* 15: 127-153.
- HEATLEY, NORMAN GEORGE 1935 The distribution of glycogen in the regions of the amphibian gastrula; with a method for the micro-determination of glycogen. *Biochem. J.* 29: 2568-2572.
- HEGNER, ROBERT W. 1923 The effects of changes in diet on the incidence, distribution and numbers of certain intestinal protozoa of rats. *Am. J. Hyg.* 3: 180-200.
- HENRY, W. A. 1912 Table III. Average digestible nutrients and fertilizing constituents in American feeding stuffs. In "Feeds and Feeding," 12th ed. Madison, Wis., pp. 582-589.
- HUNNINEN, ARNE V. 1935 Studies on the life history and host-parasite relations of *Hymenolepis fraterna* (*H. nana*, var. *fraterna*, Stiles) in white mice. *Am. J. Hyg.* 22: 414-443.
- HSÜ, H. F. 1938 Studies on the food and the digestive system of certain parasites. II. On the food of *Schistosoma japonicum*, *Paragonimus ringeri*, *Dirofilaria immitis*, *Spirocerca sanguinolenta* and *Rhabdias* sp. *Fan Mem. Inst. Biol., Zool. Ser.* 8: 347-366.
- KERLY, MARGARET 1930 The solubility of glycogen. *Biochem. J.* 24: 67-76.
- LAPAGE, GEOFFREY 1937 Nematodes Parasitic in Animals. London. 172 pp.
- LEVINE, P. P. 1938 Observations on the biology of the poultry cestode *Davainea proglottina* in the intestine of the host. *J. Parasitol.* 24: 423-431.
- MCCOY, O. R. 1935 The physiology of the helminth parasites. *Physiol. Rev.* 15: 221-240.
- MARKOV, G. S. 1939 Nutrition of tapeworms in artificial media. *Compt. Rend. Acad. Sc. U.R.S.S. (Dok.)* 25: 93-96.
- ORTNER-SCHÖNBACH, PAULINE 1913 Zur Morphologie des Glykogens bei Trematoden und Cestodens. *Arch. Zellforsch.* 11: 412-449.
- REID, W. M. 1940 Some effects of short starvation periods upon the fowl cestode *Raillietina cesticillus* (Molin). *J. Parasitol.* 26 Suppl: 16.
- 1942 The removal of the fowl tapeworm *Raillietina cesticillus* by short periods of starvation. *Poultry Sc.* 21: 220-229.
- REID, W. M., J. E. ACKERT AND A. A. CASE 1938 Studies on the life history and biology of the fowl tapeworm *Raillietina cesticillus* (Molin). *Tr. Am. Micr. Soc.* 57: 65-76.
- SAHYUN, MELVILLE 1931 Determination of glycogen in tissues. *J. Biol. Chem.* 93: 227-234.
- SALISBURY, L. FRANK AND R. J. ANDERSON 1939 Concerning the chemical composition of *Cysticercus fasciolaris*. *J. Biol. Chem.* 129: 505-517.
- SHORB, DOYS ANDREW 1933 Host-parasite relations of *Hymenolepis fraterna* in the rat and the mouse. *Am. J. Hyg.* 18: 74-113.
- SMORODINZEW, I. A., K. W. BEBESCHIN AND P. S. PAWLOWA 1933 Beiträge zur Chemie der Helminthen. I Mitteilung: Die Chemische Zusammensetzung von *Taenia saginata*. *Biochem. Z.* 261: 176-178.
- SMORODINZEW, I. A. AND K. W. BEBESCHIN 1935 The content of glycogen in tapeworms (Cestoides). *Compt. Rend. Acad. Sc. U.R.S.S. (Dok.)* 3: 413-414.
- AND ——— 1936 Beiträge zur Chemie der Helminthen. III Mitteilung. Die Chemische Zusammensetzung des *Taenia solium*. *J. Biochem. (Japan)* 23: 19-20.

- STAFSETH, H. J. 1935 On the control of tapeworm infestation in chickens with notes on the pathology of the intestines of the hosts. Mich. Agric. Exper. Sta. Tech. Bull. 148: 1-46.
- STOLL, NORMAN R. 1936 Tapeworm studies III. Sheep parasitized with one *Moniezia expansa* each. J. Parasitol. 22: 161-179.
- VOGEL, H. AND TH. VON BRAND 1933 Über das Verhalten des Fettes in den einzelnen Entwicklungsstadien von *Fasciola hepatica* und seine Beziehungen zum Exkretionssystem. Z. Parasitenk. 5: 425-431.
- WARDLE, R. A. 1935 Fish tapeworm. Biol. Board Canada, Ottawa. Bull. 45: 1-25.
- 1937a. The physiology of the sheep tapeworm, *Moniezia expansa* Blanchard. Canad. J. Res. 15D: 117-126.
- 1937b. The physiology of tapeworms. In "Manitoba Essays," 60th Anniv. Commemor. Univ. Manitoba, Winnipeg. Macmillan, pp. 338-364.
- WEINLAND, ERNST 1901 Über den Glykogengehalt einiger parasitischer Würmer. Z. Biol. 41: 69-74.
- WETZEL, R. 1932 Zur Kenntnis des weniggliedrigen Hühnerbandwurmes *Davainea proglottina*. Arch. Wissensch. u. Prakt. Tierh. 65: 595-625.
- 1934 Untersuchungen über den Entwicklungskreis des Hühnerbandwurmes *Railletina cesticillus* (Molin, 1858). Arch. Wissensch. u. Prakt. Tierh. 68: 211-232.
- WOODLAND, W. N. F. 1924 On the life cycle of *Hymenolepis fraterna* (*H. nana* var. *fraterna* Stiles) on the white mouse. Parasitology 16: 69-83.

HYPODECTES CHAPINI N. SP. (ACARINA) FROM
THE RED-SHAFTED FLICKER

G. M. SPURLOCK AND J. T. EMLÉN, JR.

Division of Zoölogy, University of California at Davis*

A red-shafted flicker (*Colaptes cafer collaris*) captured February 16, 1940, at Davis, California, and held in captivity until April 4, showed, on autopsy, 202 specimens of an immature acarian loosely encysted in the connective tissue surrounding the trachea, and, to a lesser extent, the esophagus. The greatest concentration was near the point of entrance of the trachea into the buccal cavity, but the area of infection extended from the lungs to the tongue. Another flicker, brought directly from the field on April 5, showed essentially the same picture, although the infection was not so heavy.

No movement was visible within the cysts as far as could be determined, and there was no apparent pathology in the host tissue at the site of the infection. When the parasites were placed in water the only definite movements were the slow waving of the legs, although the abdominal constriction appeared to become more pronounced in some individuals after a few minutes. No locomotion was observed in water on a tissue or glass surface.

These mites have been identified as a new species of the genus *Hypodectes* Filippi (1861). Giebel (1861) published descriptions of certain mites that had been collected by Nitzsch from the internal tissues of birds, erecting for them the genus *Hypoderas*. Later (1871) Giebel pointed out that Filippi's genus *Hypodectes* and the genus *Hypoderas* were synonymous but maintained that the generic name *Hypoderas* should be retained because it was an older genus (*Hypoderas* had been an unpublished manuscript name of Nitzsch) and because Filippi had named only 1 species in his genus (actually 5 species were named) while *Hypoderas* was supported by 12 species. Giebel also objected to Filippi's procedure of naming *Hypodectes nycticoracis* after the host, since *Ardea nycticorax* shelters two species of the genus.

A comparison of the dates of publication of the original descriptions of these two genera shows that Filippi published in June, 1861, while Giebel's paper appeared in October–November of the same year. The present authors therefore consider that the correct generic name should be *Hypodectes* and that Giebel's reasons for retaining the name *Hypoderas* were inadequate.

Received for publication, November 14, 1941.

* The writers are gratefully indebted to Dr. M. A. Stewart, Dr. R. L. Usinger, and Dr. T. I. Storer for suggestions and criticisms.

Robin and Ménézin (1877) published observations on the life history of *Hypodectes columbae* Slosarski found in pigeons and showed that it was a hypopial nymph of *Pterolichus falciger*, a feather mite, now called *Falculifer rostratus* Buchholz. According to these authors, the normal nymph molts into the hypopial stage during adverse environmental conditions, as during molting of the bird, etc. The hypopial forms migrate internally through feather follicles or respiratory organs of the bird host and come to lie in subcutaneous or tracheal tissue, there to live and grow until such time as external conditions become normal when they return to the outside and take on their original form. This deviation of the normal life cycle is supposed by these authors to be a means of saving the colony from destruction.

In view of the findings of Robin and Ménézin (*loc. cit.*) the genus *Hypodectes* probably has no true taxonomic standing, but like the helminth genera *Cercaria*, *Cysticercus*, *Microfilaria*, etc., it is used to designate immature forms for which the adult stages may be unknown.

Since the life history of the present form has not been studied it seems advisable to establish a new species for its reception until such time as the taxonomic position of the adult stage has been definitely determined.

The authors take great pleasure in naming this species after Dr. E. A. Chapin who has been kind enough to refer us to information on the affinities of this species.

Hypodectes chapini n. sp.

(Fig. 1)

General appearance white and smooth with brownish sclerotized areas; measurements 0.54 to 1.15 mm long by 0.22 to 0.42 mm wide (through middle of cephalothorax); anterior end abruptly truncate; end of abdomen evenly rounded. Cephalothorax convex dorsally and flattened ventrally; triangular area with sclerotized margins present on anteroventral surface; no trace of anterior body opening, sensory papillae, or penetrating apparatus. Mouth-parts absent. Legs, eight in number, two pairs anterolaterally and two pairs posterolaterally on ventral side of cephalothorax. Four distinct segments plus a terminal appendage on all legs; basal segment largest; terminal appendage with one long and one or two short hairs. Legs articulate to sclerotized supports on body wall. Small shelf-like projection of body wall dorsal to each posterior group of legs. Only body opening detected, a median slit with two lateral lips connected by muscle fibers to body wall, located in medio-ventral line between fourth pair of legs. Abdominal constriction, between cephalothorax and abdomen, very marked in some specimens so that abdomen appears heart-shaped; constriction less distinct in others. Abdominal constriction traversed ventrally by one large and in some specimens one or two pairs of small sclerotized areas which appear thickened internally; sides of constriction with small indistinct sclerotized area laterally. Posterior end of abdomen with sclerotized cap traversing abdomen ventrally, also dorsally to abdominal constriction; abdomen, like cephalothorax, rather flattened ventrally and convex dorsally. Degree of sclerotization variable, some individuals with little trace of terminal sclerotized cap or triangular area and less sclerotization of leg supports; no constant correlation between body size and degree of sclerotization.

Internal structures (observed in whole mounts and by micro-dissection) are muscle fibers on body wall, and many large cells containing spherules and granules

of various sizes; large cells appear to contain much fatty material. Digestive tract lacking. Muscle fibers extend from body wall to lips of body opening and to legs. Cuticle of body wall smooth externally, but covered on inner surface with many small tubercles and ridges, especially in areas of heavy sclerotization.

Host.—*Colaptes cafer collaris* Vigors (Red-shafted flicker).

Location.—Connective tissue cysts on trachea and oesophagus.

Locality.—Davis, California.

Type.—Type and 4 paratypes in U.S.N.M. No. 1383, remaining paratypes in senior author's collection.

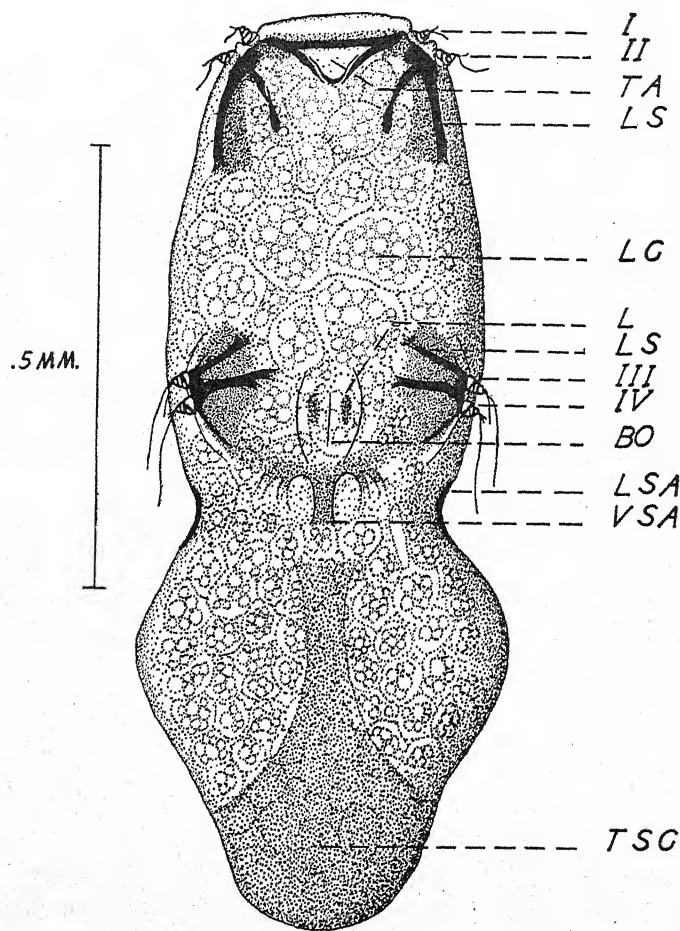


FIG. 1. *Hypodectes chapini*

Drawing by camera lucida—ventral view.

I, II, III, IV—legs

BO—body opening

L—lip of body opening

LC—large cell

LS—leg support

LSA—lateral sclerotized area

TA—triangular area

TSC—terminal sclerotized cap

VSA—ventral sclerotized area

Hypodectes chapini may be distinguished from the other species of the genus by the abdominal constriction behind the last pair of legs, the lack of body setae except on the legs, and the sclerotization pattern of the body.

The genus *Hypodectes* has been reported from the United States in domestic pigeons by Garman (1884), Kellicott (1892), and Ward (1894), and from the little blue heron (*Florida caerulea*) by Leidy (1890). Beebe (1902) reported mites (apparently of the genus *Hypodectes*) from subcutaneous tissue on each side of the keel of the sternum in the white ibis (*Guara alba*) in the New York Zoological Park. He also noted similar forms in the little blue heron (*Ardea caerulea*), the great-crowned pigeon (*Goura coronata*), the Nicobar pigeon (*Calaenus nicobarica*), and the roseate spoonbill (*Ajaja ajaja*). This author ascribes mortality among these birds to the presence of the mites. Ward (1902) doubts that the mites were the cause of mortality in these instances. Several of the bird hosts noted by Beebe (1902) were already known to harbor various species of *Hypodectes*, a fact of which Beebe was apparently unaware.

Dr. E. A. Chapin states (personal correspondence) that he has recovered specimens of *Hypoderas* (= *Hypodectes*) several times from post-mortem examination of wild birds.

Hypodectes chapini n. sp. is evidently the first member of this genus that has been recorded for *Colaptes cafer collaris* and the second species to be specifically identified in North American birds, the first being *H. columbae* mentioned above.

BIBLIOGRAPHY

- BANKS, N. 1915 The Acarina or mites. U. S. Dept. Agric. Rep. No. 108, pp. 1-153.
- BEEBE, C. W. 1902 Preliminary observations on a subdermal mite occurring among the birds in the New York Zoological Park. *Science* 15: 754-755.
- DE FILIPPI, FILIPPO 1861 Note zoologiche di Filippo de Filippi. I. *Hypodectes* nuovo genere di acaridi proprio degli uccelli. *Arch. Zool., Anat. e. Fisiol.*, 1: 52-60.
- GARMAN, H. 1884 *Pterolichus falciger*, Mégnin, observed in the United States. *Am. Naturalist* 18: 430-431.
- GIEBEL, C. 1861 Die Milbenarten der Gattung *Hypoderas* Nitzsch. *Z. Ges. Naturw.* 18: 438-444.
- 1871 Ueber einige Milben. *Idem* 27: 29-32.
- KELLICOTT, D. S. 1892 *Hypoderas columbae*. A note. *Insect Life* 5: 77-78.
- LEIDY, J. 1890 *Hypoderas* in the little blue heron (*Florida caerulea*). *Proc. Acad. Nat. Sc., Phila.* 1890, p. 63.
- ROBIN, CH. AND P. MÉGNIN 1877 Mémoire sur les sarcoptides plumicoles. X. Description particulière des genres et des espèces de sarcoptides plumicoles. *J. Anat. et Phys.* 13: 391-429.
- WARD, H. B. 1894 On the pigeon mite *Hypodectes* Filippi. *Pysche* 7: 95-100.
- 1902 The subdermal mite occurring among birds. *Science* 15: 911.

RESEARCH NOTES

A MODIFICATION OF ZINC SULFATE CENTRIFUGAL FLOTATION METHOD FOR RECOVERY OF HELMINTH OVA IN FORMALINIZED FECES

The zinc sulfate centrifugal flotation technic (Faust et al, 1938, Am. J. Trop. Med. 17: 79-84) was substituted for the Willis brine levitation technic in order to detect both protozoan cysts and helminth ova. This was done at a time (Sept., 1940) when formalinized fecal specimens were being examined in this laboratory. In subsequent fecal examinations zinc sulfate CF was found to yield a low incidence of hookworm in specimens coming from regions of this state where a high hookworm incidence was known to be present.

In addition, zinc sulfate CF as originally described was found to be excessively time consuming in this laboratory where fifty to seventy-five thousand fecal specimens are examined annually.

Different modifications of the zinc sulfate CF method were tested and the results compared with those obtained by the Willis' brine levitation method. Every formalinized fecal specimen was thoroughly comminuted and divided into two equal portions before being submitted to any diagnostic procedure.

What was considered to be a satisfactory modification of the zinc sulfate CF method was found, and this paper presents the results obtained with the original method and the modified method. In each case the results were compared with those obtained by Willis' brine levitation.

The following is a brief description of the methods used in this study:

(1) The zinc sulfate CF using zinc sulfate solution of specific gravity 1.180; the preparations of feces were strained through cheesecloth, washed two times, and the surface layer material recovered by the superimposed coverslip method. This is the same as that described by Faust et al (1938).

(2) Willis' brine levitation. The emulsified specimens were poured into small metal boxes, the latter filled with brine specific gravity 1.200 and a large glass fecal slide superimposed over each box. After five or ten minutes these slides were examined.

(3) A modified zinc sulfate CF similar to No. 1, except that a zinc sulfate solution specific gravity 1.200 was used and cheesecloth straining and the second washing were omitted. The surface layer material was removed by the superimposed coverglass method.

Centrifugation time was the same in both centrifugal flotation procedures.

A preliminary series of 100 formalinized specimens was examined by methods (1) and (2). These were thoroughly comminuted and divided into two equal portions. All had been in contact with 3-4 per cent formalin for about two weeks, and were from a region of high hookworm incidence. Method (1) revealed 10 (10 per cent) positives for hookworm and method (2), 60 (60 per cent) positives. Method (1) did not reveal any positives not found by method (2).

A second group of 1,222 formalinized fecal specimens obtained from a wide variety of places in Florida was examined by methods (2) and (3). The results of this series are presented in the accompanying table.

TABLE 1.—Incidence of helminth infections revealed by methods (2) and (3) in 1,222 fecal examinations

Eggs of	NaCl. lev. No. (2)		ZnSO ₄ CF No. (3)		Total incidence
	Number	Per cent	Number	Per cent	
Hookworm	407	33.3%	403	32.1%	470
Ascaris	25	2.0%	31	2.4%	31
Whipworm	10	0.8%	7	0.6%	12
Pinworm	9	0.7%	9	0.7%	15
Tapeworm (<i>H. nana</i>) ..	0	0.0%	2	0.1%	2
Total helminths	451	36.9%	452	37.0%	530

In the first series of 100 fecal examinations no positive specimens were found by zinc sulfate CF that were not found by Willis' brine levitation. Thus 60 represents the total incidence for hookworm, the brine revealing 50 more than the zinc sulfate CF.

In the second series of 1,222 this was not the case. Zinc sulfate revealed 340 (a) cases of hookworm that were also found positive by the Willis' brine levitation method. The Willis' brine levitation revealed an additional 67 (b) cases missed by zinc sulfate CF. Thus Willis' brine levitation revealed (a) plus (b) (340 plus 67), or 407 positives. On the other hand, zinc sulfate CF revealed hookworm in an additional 63 (c) cases missed by brine. Zinc sulfate revealed (a) plus (c) (340 plus 63), or 403 positives. Thus, (a) plus (b) plus (c) (340 plus 67 plus 63) equals 470, or the total number of specimens positive for hookworm in the 1,222 specimens examined by these two methods.

The increased efficiency as a result of the modification of the zinc sulfate CF is obvious. In the first 100 specimens the Willis brine method found 50 per cent more hookworm than the zinc sulfate CF, while in the latter 1,222 specimens, the brine recovered only 1.2 per cent more hookworm than zinc sulfate CF. Furthermore, the time required to perform method (2) was less than half that required for method (1).—WILLIAM A. SUMMERS, Senior Parasitologist, Bureau of Laboratories, Florida State Board of Health, Jacksonville, Florida.

THE PERIODICITY OF OÖCYST DISCHARGE IN COCCIDIAL INFECTION OF CHICKENS

The periodicity of oöcyst discharge in sparrows and pigeons affected with coccidiosis has been demonstrated by Boughton (1932, J. Parasitol. 19: 177; 1934, Ibid. 20: 329; 1937, Ibid. 23: 291-293). The experiments described herein were performed to determine whether or not a similar phenomenon characterized the development of coccidial oöcysts of the chicken.

Coccidia-free White Leghorn males, seven weeks old, were used. Five groups of nine to ten birds each were dosed into the crop with suspensions of sporulated oöcysts of *Eimeria praecox*, *E. mitis*, *E. maxima*, *E. hagani* and *E. necatrix*, respectively, in the month of July. The birds in each group received the same number of oöcysts, varying from 800 of *E. maxima* to 8,900 of *E. necatrix*. By mistake, the dose of *E. hagani* was much larger (300,000). The doses were kept small purposely to avoid producing severe infections which would alter the feed intake, character of the feces, etc. *E. hagani*, a non-pathogenic species, had no effect on the birds despite the size of the dose. Each group was dosed at 9 AM at a date so chosen that the first discharge of oöcysts resulting from the infection occurred on the same day in every group. In this way there was assured a uniformity in the environmental influences that might have had an effect on the oöcyst discharge of the different species. Sterilized holding batteries with wire screen floors and removable dropping pans housed the five groups. An adequate all mash ration was fed. Feces of each group were collected and weighed at 9 AM, 3 PM and 9 PM. Oöcyst counts were made according to the method already described (Levine, 1939, Cornell Vet. 29: 309-320). The total number of oöcysts discharged and the percentages of the total daily oöcyst and fecal discharges for each of the three periods of the 24 hours were computed and graphed. *E. acervulina* was not available at the time this study was made. *E. tenella* was not used since infection with this species, in many instances, causes the formation of a caseous plug in the cecal lumen, thereby preventing the discharge of oöcysts from that organ.

The results are indicated in the series of graphs in Fig. 1. The amount of feces collected during each of the three periods of the day was about the same. There was a tendency for a slight increase during the 9 AM to 3 PM interval. The discharge of oöcysts, however, fluctuated in a periodic manner, although the steepness of the curves for each of the species was not the same. The most marked periodic discharge was shown by *E. hagani*. During the six hours from 3 PM to 9 PM, 87% to 91% of the total daily output was eliminated. An insignificant num-

ber of oöcysts were eliminated during the rest of the day. Two other trials with *E. hagani* on different occasions yielded similar results. None of the other species discharged so many oöcysts in such a short time. The number of oöcysts of *E. praecox* reached a peak during the same time interval but the discharge during the

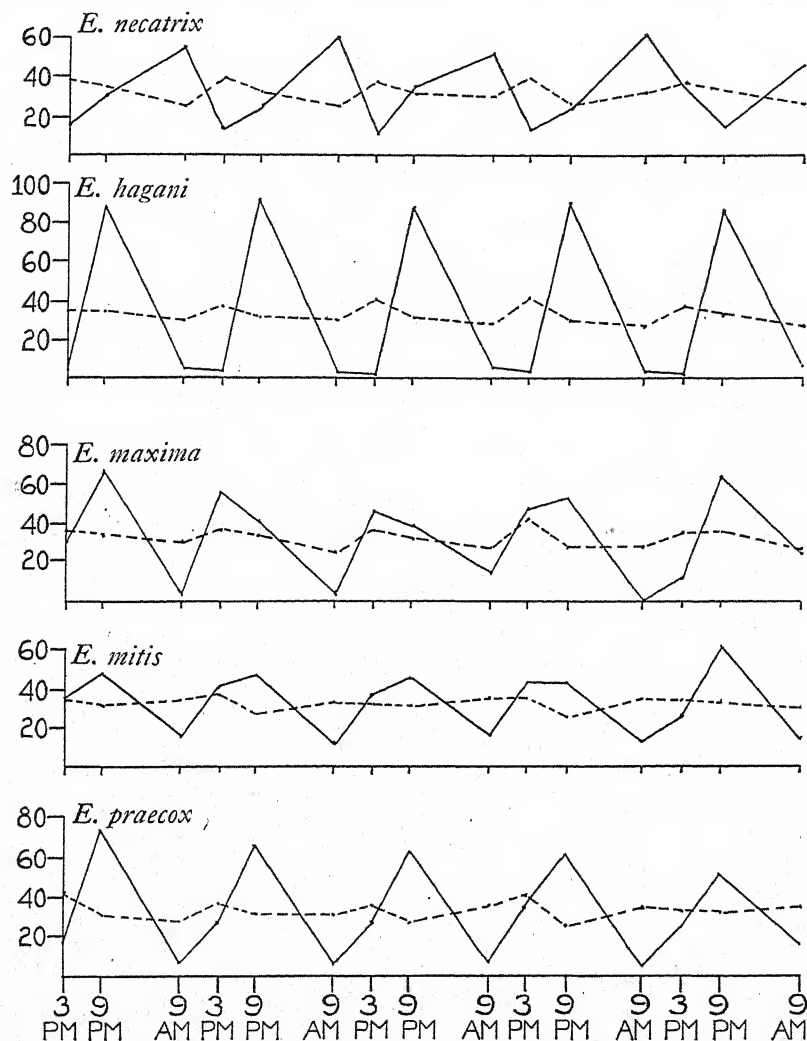


FIG. 1. Periodic fluctuations in the percentages of oöcysts (solid line) and feces (broken line) discharged by experimentally infected birds during the periods between 9 AM-3 PM-9 PM.

rest of the day accounted for from 26% to 43% of the total. The same was true for *E. mitis* except that the curve was flattened still more, since from 37% to 55% of the oöcysts were discharged during 9 PM to 3 PM the following day. The results with *E. maxima* were somewhat erratic since the peak of the curve was not reached during the same period each day. On three days, the greatest number was discharged during the 3 PM-9 PM interval while on two days the peak was reached

between 9 AM and 3 PM. With the exception of the last day, the discharge of oöcysts of *E. maxima* was smallest during the 9 PM-9 AM period. *E. necatrix* differed from all the other species in that the greatest oöcyst discharge occurred during the night and early morning, between 9 PM and 9 AM. From 47% to 62% of the oöcysts were eliminated during this time. One cannot conclude, however, from examination of the graph, that the formation of oöcysts of *E. necatrix* occurs periodically even though the apparent discharge makes it appear so. The oöcysts of this species are produced in two cecal pouches. These organs not only empty themselves independently of one another, but also independently of the rest of the intestinal tract. Consequently, until some way is found to collect the cecal discharges separately from those of the intestine, it will be impossible to determine whether there is true periodicity in this species.

Undoubtedly, the shape of the curves might have been different had this study been made during another time of the year when the number of hours of daylight are smaller, or had other time intervals been chosen. However, since all of these trials were run simultaneously, the basis for comparison is identical. The periodicity of *E. hagani* is so characteristic, that it may be used as another criterion for the identification of this species in addition to those described previously (Levine, 1938, Cornell Vet. 28: 263-266).—P. P. LEVINE, *Department of Pathology and Bacteriology, New York State Veterinary College, Ithaca, N. Y.*

PARASITOLOGICAL SURVEY OF LOUISIANA MUSKRATS

This paper contains a general report of the parasites of the Louisiana muskrat (*Ondatra zibethica rivalica* Bangs), with notes on the incidence of some of the species found. All of the muskrats were trapped in 1939-40 and 1940-41 in several localities along the Louisiana coast. Principal among these is the Willow Bayou area of the Sabine National Wildlife Refuge in Cameron Parish, the Vermilion Bay area in Vermilion Parish, and the Michaud marsh in Orleans Parish.

In addition to Ernest C. Martin for collaboration on parts of this study, the writer is indebted to Dr. Carl E. Venard, Ohio State University, and Dr. J. E. Shillinger, U. S. Fish and Wildlife Service, respectively for identifications of the pentastome and most of the helminths; to Florence Brooks Kreeger and Dr. Harley N. Gould, Tulane University, for many of the carcasses examined; and, to Harry E. Adams, Julian A. Howard and others of the Sabine National Wildlife Refuge personnel for cooperation during the course of the work.

Of the total of 1,780 muskrats examined in the past two trapping seasons, only 53 were more or less completely searched for all parasites; the remainder were examined for only several particular species. All autopsies were made in the laboratory from freshly-skinned carcasses as they were secured from the trappers.

Because of their semi-aquatic habitat, natatorial predilections, and often heavy concentrations one would expect muskrats to be subject to infections by large numbers of helminths, especially trematodes, and other parasites. Svihla and Svihla (1931, J. Mamm. 12: 12-18) have reported that muskrats of Louisiana were practically free of parasites.

EXTERNAL PARASITES

Probably because of the condition of the marshes at the time of the studies, during which rainfall was good and the water level at an optimum depth in the runways, very few external parasites were observed.

Mites—*Tetragnonyssus spiniger* Ewing & Stover were most abundant in the nests of the young muskrats. At Willow Bayou they were present on about one-fourth of the muskrats, but there were seldom more than one or two to the animal. Trappers in all parts of the state report that in drought years the muskrats become very heavily infested with mites and that at such times their own clothes must be changed frequently to get rid of the "vermin"; these mites, however, seem to die soon after leaving the fur of the host. Svihla and Svihla (1931) noted mites on muskrats at Avoca Island near Morgan City, St. Mary Parish, La.

Diptera.—Several larvae of the flesh-fly, *Sarcophaga* sp., were found in the fur of two muskrats from Willow Bayou.

INTERNAL PARASITES

Protozoa.—Two species were found in the intestines of some muskrats. *Giardia*, apparently the recently described *G. ondatrac* (Travis, 1939, J. Parasitol. 25: 11-17), was of common occurrence in all of the intestinal smears examined, and small flagellates, probably *Trichomonas* sp., were also found in several duodenal smears.

Nematodes.—*Capillaria hepatica* (Bancroft) occurred in 10 percent of Michaud muskrats, nearly 50 per cent of Vermilion Bay 'rats, and was completely absent at Willow Bayou. *C. hepatica* has been recorded from muskrats also by Price (1931, J. Parasitol. 18: 51) and Warwick (1936, Parasitology 28: 395-401).

Rictularia sp. and *Physaloptera* sp. were found in small numbers in about one-fourth of both the Michaud and Willow Bayou animals. Chandler (1941, J. Parasitol. 27: 175-181) has just described *Rictularia ondatrac* from muskrats in south-eastern Texas.

Longistriata adunca was found in one muskrat from Willow Bayou.

Tapeworms.—Only two were encountered, both from Willow Bayou, and have been identified as *Hymenolepis evaginata*.

Trematodes.—In spite of the tremendous number of flukes encountered in the small intestine of the Michaud muskrats, apparently only three species were present. The most common by far was the small monostome *Nudacotyle novicia* Barker. In some muskrats they covered the walls of the intestine, very large numbers being recovered in zinc sulfate flotations. This species, however, was of rare occurrence in the Willow Bayou area, nor did Chandler (1941) find it abundant in east Texas.

Echinochasmus schwartzi Price was of high incidence at Willow Bayou, but rare at Michaud. This species was the most abundant of those found by Chandler (1941) in east Texas.

A few specimens of *Paramonostomum pseudalveatum* Price, were found in a single muskrat from Willow Bayou.

Pentastomes.—A nymphal pentastome of the family Porocephalidae, *Porocephalus crotali* (Humboldt), infecting mainly the liver and lungs occurred in 9% of the muskrats from the whole state. Infection by this parasite has been studied in detail and is reported elsewhere (Penn and Martin, 1941, J. Wildlife Management 5: 13-14; and Penn, 1942, J. Parasitol. In press).

SUMMARY

Thirteen species of parasites are recorded from Louisiana muskrats. These include one mite, one flesh-fly larva, two flagellated protozoa, four nematodes, three trematodes, one tapeworm, and one nymphal pentastome.—GEORGE H. PENN, JR., Wildlife and Fisheries Division, Louisiana Department of Conservation, New Orleans.

COCHLOSOMA ROSTRATUM FROM THE TURKEY

In 1923 Kotlan (Centr. Bakt. I Abt. Orig. 90: 24-28) described as a new genus, *Cochlosoma*, a flagellate found in the cecum of European domestic ducks. He named this species *Cochlosoma anatis*. He found it in ducks which showed inflammation of the intestinal tract.

In 1934 Kimura (Tr. Am. Micr. Soc. 53: 102-115) found a representative of the same genus in Muscovy and white Pekin ducks in California. Certain morphological characteristics, principally the shape of the sucker, convinced him that it was a new species, which he named *Cochlosoma rostratum*. He found it to be widespread in occurrence in ducks in this state, although more abundant on ranches than in market birds. *Cochlosoma rostratum* measures 6-10 × 3.9-6.7 microns. The most characteristic organelle is the ventral sucker which is half the length of the body and in fresh preparations reminds one of *Giardia*, although the curvature and

depth of the sucker is more pronounced. "The characteristic movement is constant circling in a very small radius with a spiral twisting of the body." This is in contrast to the jerky movement of *Trichomonas*, or to the rapid darting motion of *Hexamita*.

In our studies of infectious catarrhal enteritis (hexamitiasis) of turkey poults (1938, Cornell Vet. 28: 281-293) we have observed a flagellate which is indistinguishable morphologically from *C. rostratum*. In young poults we have found it throughout the intestinal tract, and in adults in the region of the cecal tonsil. Kimura found it usually only in the large intestine and occasionally in the lower ileum of ducks. Unlike Kotlan he felt that it was of no pathogenic significance. Since the age of the ducks Kimura examined is not clear, it is possible that in ducklings the organism may be found throughout the intestine. In turkey poults we have always found it associated with *Hexamita* or in combinations of *Hexamita* and *Salmonella*.

The true significance of this parasite in turkey poults or ducklings has not been determined.—E. McNEIL AND W. R. HINSHAW, *University of California, Division of Veterinary Science, Davis, California*.

A SEVEN-YEAR-OLD INFECTION OF *CRYPTOCOTYLE LINGUA* CREPLIN IN THE WINKLE *LITTORINA LITTOREA* L.

In 1935 I collected several hundred specimens of *Littorina littorea* L. One of the largest of these molluscs, measuring 33 mm in length, was infected with the trematode *Cryptocotyle lingua* Creplin. It has been kept by me since that date in filtered sea water, and is incidentally the only one of my experimental animals to survive the Plymouth "blitz." In a letter (Nature, 1940, 146: 367) I recorded that approximately 5,500,000 cercariae had emerged during five years, and in August 1938 the mean daily count was still 830. Curiously this figure has now risen to approximately 1,600 cercariae per 24 hours.

As filtered sea water has been used throughout the experiment, any possibility of re-infection is precluded. I think, therefore, it is worth while drawing attention to the fact that a single infection has survived nearly seven years and is still producing thousands of cercariae. This is in itself no evidence against the current theory of germinal lineage, to which many helminthologists subscribe, but the implications are interesting, and it certainly raises several points relating to the phenomenon which require investigation.—MIRIAM ROTHSCHILD, *Ashton Wold, Peterborough, England*.

The Journal of Parasitology

Volume 28

OCTOBER, 1942

Number 5

NOTES ON THE MUSCULATURE OF THE MALE GENITALIA OF *HAEMONCHUS CONTORTUS*

W. L. THRELKELD AND M. E. HENDERSON

Virginia Agricultural Experiment Station, Blacksburg, Virginia

In connection with certain unpublished studies dealing with gametogenesis in *Haemonchus contortus*, mass sectioning of material under study revealed several longitudinal sections in dorso-ventral sequence, of the posterior region of the male worm. While the anterior portion appears slightly below the level of the more posterior region and the whole section is slightly tilted toward the left (thus rendering structures on the right more prominent to the observer) nevertheless, an unusually good picture of the organs involved is presented. Cross sections through this region from the posterior intestinal glands to the cloaca inclusive were photographed. These show the relationship of the organs herein described.

Fig. 21, Plate V is a photomicrograph showing the position of the spicules upon extrusion. It is here seen that the anterior portions of the spicules are in juxtaposition with the posterior intestinal glands (I. Gl.). Such a position of the spicules would place that portion of the spicules as indicated by the arrow, Fig. 7, Plate II, very near the opening of the intestino-germinal canal shown by the arrow in Fig. 20, Plate IV, formed by the junction of the vas deferens and intestine as shown by the arrow in the sagittal sections, Fig. 22, Plate V. The sections were cut at seven microns and stained in Heidenhain's iron hematoxylin.

The muscles involved in the retraction and extension of the spicules and the operation of certain bursal rays together with the location of glandular structures along the intestine and also the position of the posterior region of the vas deferens were photographed. Camera lucida drawings were made, and the above-mentioned structures are herein briefly described.

Fig. 1, Plate I, shows the gubernaculum and the dorsal ray. The gubernaculum is strongly supported and held in place by longitudinal and transverse muscles as shown in Fig. 11, Plate III. Springing from each side of the gubernaculum is a thin cuticular structure which completely encircles the spicules and separates them from the intestine.

Received for publication, August 14, 1941.

Fig. 2, Plate I, shows a more ventral section. Here is seen a portion of the gubernaculum and the spicules faintly defined on each side of and anterior to the gubernaculum.

Fig. 3, Plate I, depicts a further ventral section just below the gubernaculum. The right spicule is more prominent than the left. The thickened recurved shanks of the spicules slightly overlap. The vas deferens appears near the bulge of the shanks of the spicules and proximal to the grooves which characterize the shanks. The spicules terminate in a heavily muscled sheath.

Fig. 4, Plate I, is a still more ventral section. Portions of the heads of the spicules are visible together with the muscles which operate the spicules. Parts of the cloacal muscular system and one pair of glands situated anterior to the cloaca are seen.

In Fig. 5, Plate I, the cloacal dilator muscles are more evident, and posterior to the gland described in Fig. 4 there may be seen two additional glands.

Fig. 6, Plate I, shows the cloacal dilatory muscles and the intestine midway between these muscles. The anterior portion of the intestine is constricted, whereas the more posterior portion is dilated and empties into the cloaca wherein may be seen the ends of the spicules.

Fig. 7, Plate II, is a camera lucida drawing of the section appearing in Fig. 4 to which were added the essential structures appearing in sections 2, 3, 5, and 6, and affords a basis for the interpretation of the function of the structures already presented. These functions are defined primarily on their various anatomical locations, origins, and insertions.

The origins of muscles 1 and 3, *Retractor spicularis lateralis* (1 and 3), could not be definitely determined, but apparently they arise laterally from the body walls of the worm. They are inserted on the heads of the spicules and their function is to retract the spicules.

Muscle 2, *Retractor spicularis centralis* (2), apparently arises from the dorsal wall of the body of the worm and proceeding caudally bifurcates into two smaller portions which insert on the heads of the spicules. Its function is to retract the spicules.

Muscle 4, *Protractor spicularis*, and its opposite fellow running from the heads of the spicules to well within the bursal lobes arises from the inner walls of the rays of the lobes and proceeds cephalad. It receives two muscular serrations arising laterally from the wall of the body before it inserts along the lateral anterior one-third of the spicule and on the head of the spicule. Its function upon contraction is to cause protrusion of the spicule and probably assists in contraction of the bursa. The caudal ends of the spicules are encased in a cuticular and heavily muscled sheath. This latter seems also to include ventrally the terminal portion of the conventionally defined cloaca.

Muscle 5, *Dilator cloacae*, and its fellow on the opposite side arise from the walls of the body and insert on the cloaca, the cuticular body wall and spicule sheath. The function of these two muscles is to dilate the cloaca and the cloacal aperture.

Muscle 6, *Bursa expansa*, lying along the floor of the caudal wall of the worm arises from the rim of the cuticular opening of the cloaca and inserts on the lateral body wall and the inner rim of the exterior wall of the bursal rays. Its function is to expand the bursa.

It seems, from a study of the materials here presented, that there are more than one of the muscles 5 and 6 as these muscles or remnants of these muscles appear in five different sections.

Fig. 9, Plate II, is a photomicrograph $\times 900$ of the thickened shank and posterior portion of the spicules as seen in Fig. 3. Within the grooves of the shank are the sperms enclosed in their gelatinous envelopes.

The camera lucida drawing, Fig. 8, including the same sectional area as that shown in Fig. 9 shows in addition a portion of the vas deferens from which a sperm has been dislodged.

The sperms lying within the grooves of the spicules were seen in sections on other slides. In one particular instance the spicules were not cut by the sectioning knife but were entirely displaced from their surrounding structures, revealing a clearer picture of the position of the sperms than the one here presented. Cross sections through the anterior rectal glands (Figs. 10 and 11, Plate III) show the muscular support of the gubernaculum and the anatomical relationship of the gubernaculum, spicules, spicular sheath, vas deferens and intestine. In a series of cross sections, Figs. 12 to 20, Plate IV, inclusive, the intestine and vas deferens evidently approach each other and join to form one canal. The lumina of these respective tubes are almost obliterated by the intimate structure of the intestinal glands which empty into the resulting canal which proceeds caudad and empties directly into the cloaca (see arrow, Fig. 20). It is at this point that sperms pass into the spicules.

DISCUSSION

Looss (3) describes three pairs of muscles of the guiding-piece (gubernaculum) and concludes his description thus: "Comparing now the various effects just described as probably produced by the six muscles of the guiding-piece it becomes obvious that by their help the tip of the latter is enabled to move, within certain limits of course, in every direction, a capability which is no doubt of importance during copulation." Similarly placed muscles are described by Looss for *Ancylostoma duodenale*. There is considerable evidence in this study, Plate III, Fig. 11, that three pairs of muscles are intimately connected with the gubernacu-

lum which could produce slight movement in three directions; namely, a cephalad-posteriad, dorso-ventral, and a lateral direction. These muscles, however, could not be traced in entirety in the material available.

The insertion of muscle 5 and the entire muscle 6, Plate II, correspond to certain muscles of the genital cone as described by Looss (3). The more anterior structures lying along the spicular sheath described in Fig. 4, Plate I, as glands are described by Looss (3 and 4) as muscle cells which give rise to rectal, sphincter muscles.

No muscle fibers arising from these structures could be discerned in this study and since they are intimately connected with the lumen of the intestine they are designated rectal glands.

Muscles 1 and 3, Plate II, are described by Looss (4) under the caption *Musculi retractores*. Muscle 2, Plate II, was not observed by Looss. Muscle 4 and its opposite fellow are described as *Musculi exertores spiculorum*. Their descriptions in general parallel the description here given with the exception that they are located by Looss (4) as originating in the crest of the cloacal wall near its anterior end instead of well within a bursal ray. The latter description would serve to assist in contracting the bursa before the spicules are extruded through the anal aperture.

Looss (4) also observed sperms on the flattened side of the spicules between the two longitudinal crests. He explains the presence of the sperms in the canal formed by the proximity of the spicules as being sperms which were not all carried out during copulation and upon retraction of the spicules were drawn back into the spicular sheaths. He further excludes the possibility that they made their way there from the point where the ductus ejaculatorius enters the cloaca. It is here suggested that in *H. contortus* the sperms enter the spicules directly from the terminus of the intestino-germinal canal and are carried in the grooves of the spicules to the vagina of the female during copulation. It would be difficult for the sperms to gain their position in the grooves of the spicules by other means since the spicules pass through an unperforated cuticular canal and are closely applied to each other upon their emergence into the cloaca. It is conceivable, however, that the spicular apparatus can be moved into a position most favorable for the reception of the sperms between the spicules. This position is well illustrated in Plate V, Fig. 21. Here the anterior end of the spicules approximates the glandular region of the rectum and brings the anterior grooved portion of the spicules opposite to the opening in the intestino-germinal canal.

SUMMARY

A study of longitudinal and cross sections through the posterior region of the male nematode, *H. contortus*, shows:

That the gubernaculum is supported and slightly activated by longitudinal and transverse muscles and that the location of these muscles implies that slight movement is possible in three directions.

Other muscles are described and their functions are explained on the basis of their origins, insertions, and locations.

a. Muscles 1 and 3, each named *Retractor spicularis lateralis*, function—to retract the spicule.

b. Muscle 2, *Retractor spicularis centralis*, function—to retract the spicule.

c. Muscle 4, *Protractor spicularis*, function—to extrude the spicule and assist in contraction of the bursa.

d. Muscle 5, *Dilator cloacae*, function—to dilate the cloaca.

e. Muscle 6, *Bursa expansa*, function—to expand the bursa.

Upon extrusion of the spicules the anterior portions of these organs wherein are the origins of the longitudinal grooves, assume a position opposite the break in the wall of the intestino-germinal canal and here receive sperms passing down through this common canal from the vas deferens.

Spermatozoa contained in gelatinous capsules are found in the grooves formed by the longitudinal spicular ridges.

The spicules conjointly act as a penis to transfer the spermatozoa from the male into the vagina of the female during copulation.

Two to three pairs of intestinal glands are present proximal to the intestine.

BIBLIOGRAPHY

1. CAMERON, T. W. M. 1934 The Internal Parasites of Domestic Animals. A and C Black Ltd., London.
2. CHITWOOD, B. G. AND M. B. 1940 An Introduction to Nematology. Section I, Part III; Section II, Part I. Babylon, New York.
3. LOOSS, A. 1901 The Sclerostomidae of horses and donkeys in Egypt. Rec. Egypt. Govt. Sch. Med. V. 1: 1-152.
4. ———— 1905 The anatomy and life history of *Agchylostoma duodenale* Dub. Rec. Egypt. Govt. Sch. Med. V. 3: 1-158.
5. MÖNNIG, H. O. 1938 Veterinary Helminthology and Entomology. William Wood and Co., Baltimore.
6. RANSOM, B. H. 1911 The nematodes parasites in the alimentary tract of cattle, sheep and other ruminants. Bull. Bureau Animal Indust., U. S. Dept. Agric. No. 127.
7. THRELKELD, W. L. 1941 Notes on copulation of certain nematodes. Virginia J. Sc. 2:

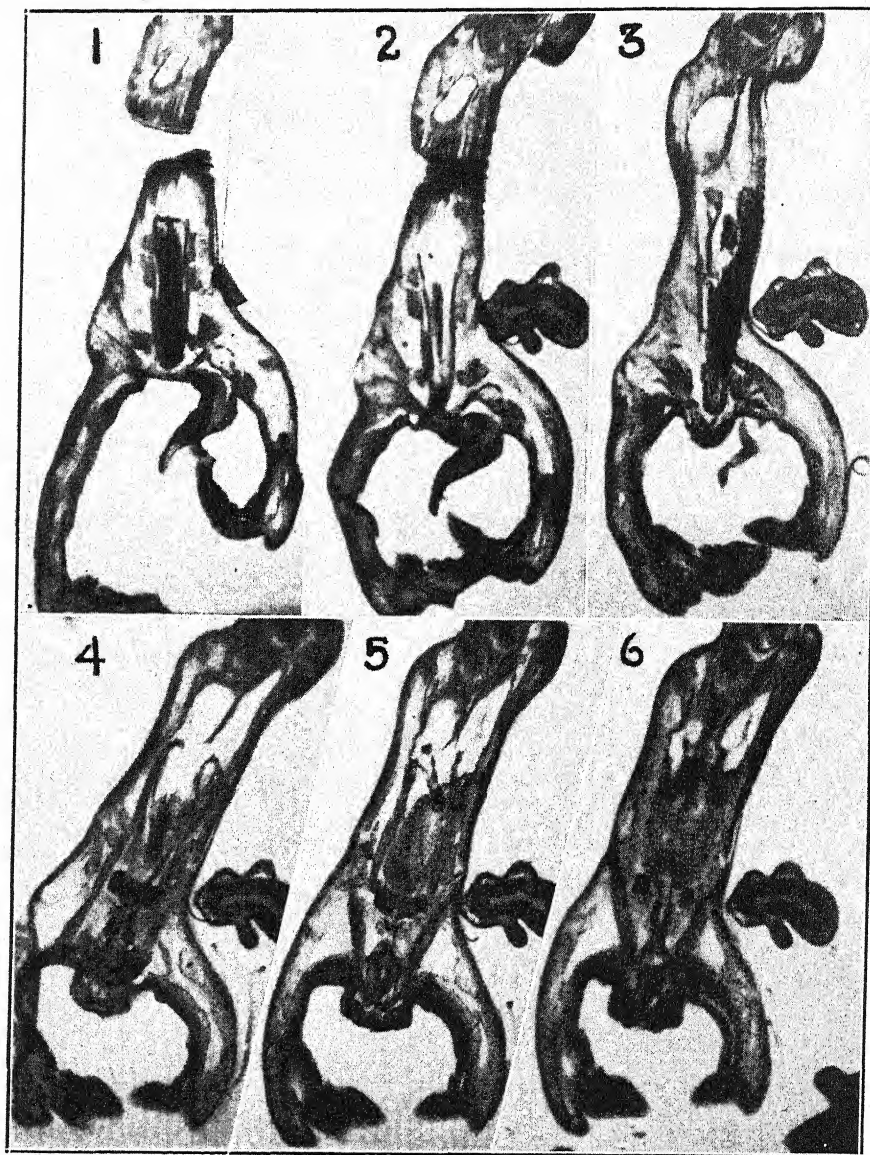


PLATE I

FIGS. 1-6. Photomicrographs $\times 100$ of dorso-ventral sections in series through posterior portion of the male *Haemonchus contortus*, showing location of organs described.

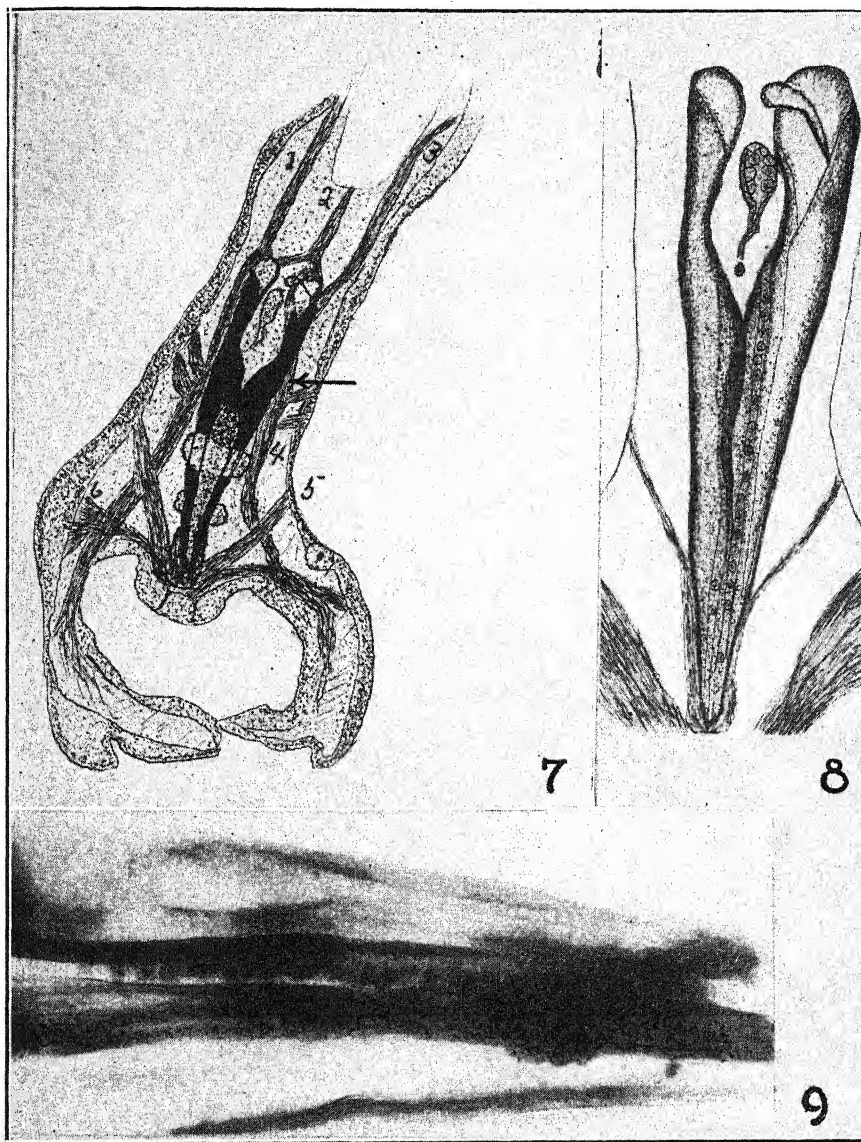


PLATE II

FIG. 7. Composite camera lucida drawing $\times 150$, reduced to $5/6$ original size, of the essential structures appearing in Figs. 2-6, inclusive.

FIG. 8. Camera lucida drawing $\times 333$, reduced to $1/3$ original size. Same section as photomicrograph, Fig. 9.

FIG. 9. Photomicrograph $\times 900$ of a portion of the spicules showing sperms in the grooves of the spicules.



PLATE III

FIG. 10. Photomicrograph $\times 900$. Transverse section through the posterior portion of a male *H. contortus*. The large black structure in the lower left hand corner of the picture is the gubernaculum. There follow in sequence the spicules, the intestine, and the glandular vas deferens.

FIG. 11. Same section as shown in Fig. 10 in slightly different plane showing support of gubernaculum by transverse and longitudinal muscles.

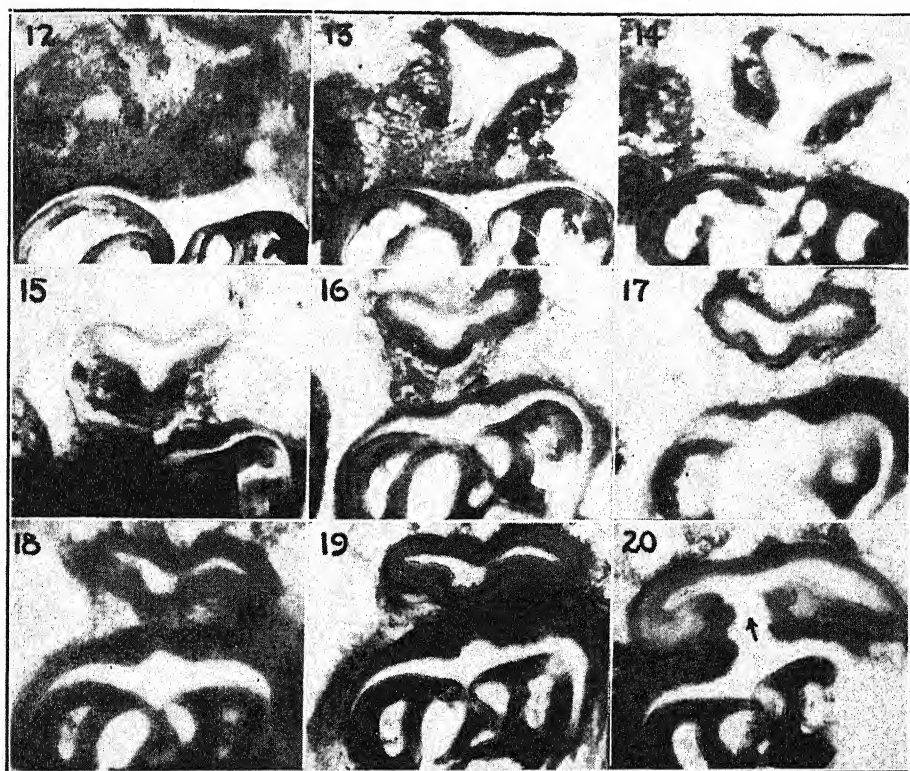


PLATE IV

FIGS. 12-20. Photomicrograph $\times 810$. Transverse sections in series from the region of the posterior intestinal glands to the cloaca showing the highly glandular portion at the merge of the intestine and vas deferens and the connection of the resulting canal with the cloaca.

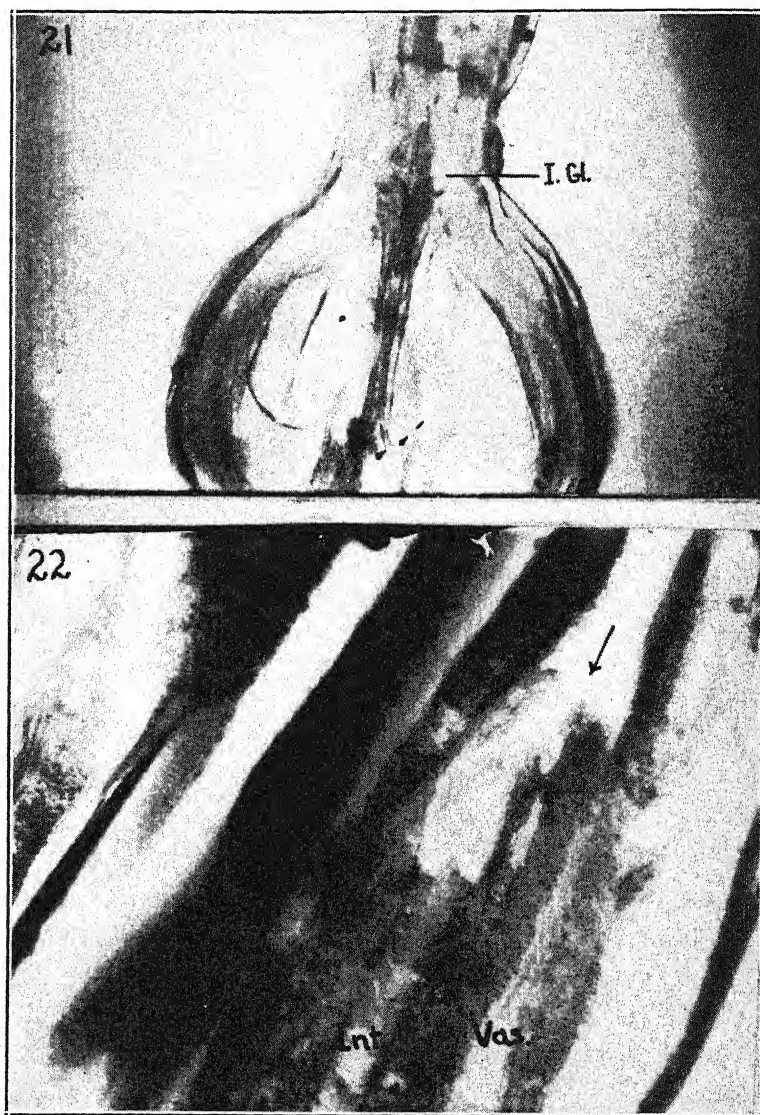


PLATE V

FIG. 21. Photomicrograph $\times 100$ showing position of spicules upon extrusion.

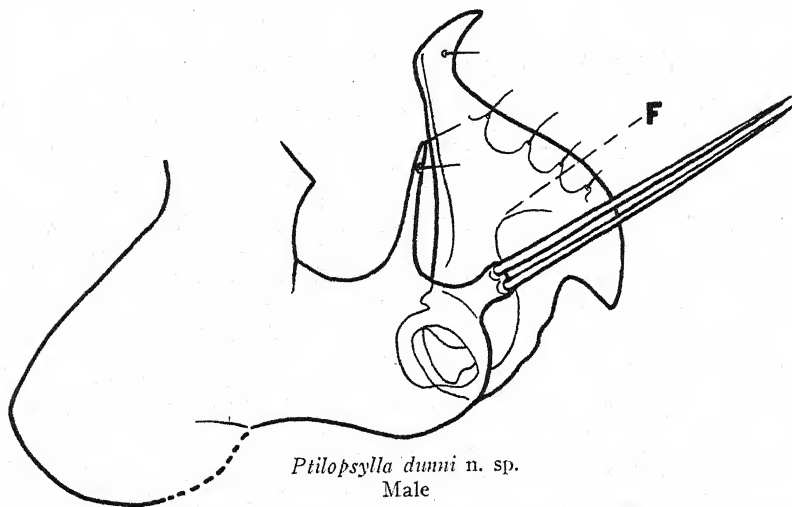
FIG. 22. Photomicrograph $\times 900$ of sagittal section showing portion of intestino-germinal canal formed by the intestine (Int.) and the vas deferens (Vas.).

SIPHONAPTERA: *PTILOPSYLLA DUNNI*, A NEW SPECIES
OF BAT FLEA FROM PANAMA¹

GLEN M. KOHLS

Associate Entomologist, United States Public Health Service

Among materials recently received for determination from the American Museum of Natural History through the courtesy of Dr. C. H. Curran, were 3 males of the genus *Ptilopsylla* Jordan and Rothschild, 1921,² collected from a bat, *Molossus coibensis* Allen, Panama City, December 6, 1933, by L. H. Dunn. The specimens were thought to represent a new species and one was sent to Dr. Karl Jordan, of the British Museum, for comparison with the only previously known species of the genus, *Pt. leptina* Jord. and Roths., 1921, which was described from a single male specimen collected from a bat at Santissima Trinidad, Asuncion, Paraguay. The writer's opinion was confirmed and Dr. Jordan stated that in the British Museum there are a male and female apparently from the same lot. Comparative notes kindly furnished by Dr. Jordan are incorporated in the description which follows.



Head: Frontal tubercle small but distinct. The two oral spines narrow, posterior one longer and broader than anterior one. Frons with an anterior row of ten bristles, the upper one longest and situated near anterior margin of antennal groove; lower bristle shorter than upper bristle but much longer than intervening ones. Ocular bristle, situated just above vestigial eye at anterior margin of antennal groove, long, reaching well beyond apex of genal process. Two other prominent bristles on frons; one near anterior margin of antennal groove between ocular bristle and upper bristle of anterior row of frons, other above base of posterior oral spine at level of frontal

Received for publication, August 11, 1941.

¹ Contribution from the Rocky Mountain Laboratory (Hamilton, Montana), of the Division of Infectious Diseases of the National Institute of Health.

² Ectoparasites 1: 158-162.

tubercle. Occiput with numerous rather stout bristles. Posterior margin antennal groove bordered with about 10 small bristles. Genal process acuminate; tip rounded off. Maxillary and labial palpi equal in length and slightly less than half length of fore coxa.

Thorax: Pronotum with comb of twenty-two curved spines; also two rows of bristles and a few scattered additional bristles. The mesopleura bear about 15 bristles. Rod dividing mesopleura in other genera obsolete but represented by remnant projecting dorsally from bar separating mesopleura from mesosternum. Metepimeron bears 14 or 15 bristles. Metanotum with two short spines on each side.

Abdomen: Tergites and sternites with strong transverse incrassations. Incrassation of basal sternite short; that of sternite VII broad, Y-shaped in lateral view. Tergite I with comb of about 8 spines, dorsal ones of which are two-thirds as long as dorsal pronotal spines. The two lower bristles of tergites II to VII widely separated from other bristles. Sternite VII has 4 or 5 bristles on each side. One antepygial bristle on each side.

Modified segments: Tergite VIII bears about 7 bristles above stigma including long bristle situated at about upper fifth of apical margin. Sternite VIII long, broad basally, narrowing gradually toward apex, upper margin nearly straight. On outside along ventral margin about 15 bristles of size found on eighth tergite; on inside a row of very closely set bristles, curved ventrad, each ending in fine wavy filament. Longer bristles are near apex and longest is about length of hind tibia. Ventroproximal margin of this sclerite is heavily sclerotized and extends to incrassation of seventh sternite. Manubrium of clasper short and broad. Clasper with two processes: upper one slender, pointed, conical, with an apical bristle and another situated subapically; lower process much shorter, truncate, somewhat dilated apically and bearing two long, strong bristles. Exopodite (F in Fig.) broad proximally, ventral margin deeply notched, lower or posterior angle pointed, apical margin rounded and upper or anterior angle drawn out into posteriorly directed pointed hook. Apical portion of ninth sternite large, conical; posterior margin slightly incurved twice, a bristle situated distally to the proximo-ventral angle and another further distad, but before the middle.

Length: Holotype, 1.8 mm (exclusive of sternite VIII).

Host: Bat, *Molossus coibensis* Allen.

Locality: Panama City, Panama.

Holotype: Male, in collection of Rocky Mountain Laboratory.

Paratypes: One male, deposited in British Museum; one male in American Museum of Natural History, New York City.

According to information received from Dr. Jordan the female agrees with the male apart from the sexual differences but has the sclerotizations of the head, thorax and abdomen less pronounced.

The species is very similar to *Pt. leptina* from which it may be most readily distinguished by the shape of the exopodite. In *leptina* the exopodite is described as "large, resembling a short-stalked mushroom; it is proximally narrow and soon widens out into a broad hood; the apical margin is rounded, the ventral (= distal) margin below the hood is deeply incurved, the anterior margin almost straight. The anterior (or upper) corner of the hood is truncate-rotundate; the posterior (or lower) corner, which is much obscured in the specimen, appears to be broadly truncate, as indicated in the figure." Also, in *dunni* tergite VIII bears about 7 bristles above the stigma including the long bristle situated at about the upper fifth of the apical margin; in *leptina* there are 11 and the long bristle is situated at about the upper third. The mesopleura bear about 15 bristles while in *leptina* there are 22 or 23.

STUDIES ON PHENOTHIAZINE. XI. THE EXCRETION OF PHENOTHIAZONE*

FLOYD DEEDS AND JOHN O. THOMAS

Agricultural Chemical Research Division, Bureau of Agricultural Chemistry and Engineering, United States Department of Agriculture, at the Department of Pharmacology, Stanford University School of Medicine, San Francisco, California

In recent years the status of phenothiazine has changed from that of a laboratory curiosity and theoretical precursor of the thiazine dyes to that of a compound with important insecticidal properties. More recently the compound has demonstrated its usefulness as a bactericidal agent in the treatment of urinary tract infections, as a fungicide, and as an anthelmintic. The oxidation products of phenothiazine are the sulfoxide, phenothiazone and thionol. Under appropriate conditions the leuco bases of the two latter compounds can be formed. Exposure of phenothiazine to air, moisture and light results in oxidation to the phenothiazone stage at least. Oxidation in the animal body occurs with even greater ease. Since some of the interesting properties of phenothiazine may be associated with the oxidation products it is desirable to record information pertaining to the fate of phenothiazine in the animal body.

In a previous paper on the fate of phenothiazine it was reported by DeEds, Eddy and Thomas (1) that the gastric administration of phenothiazine to rats, rabbits and humans resulted in the urinary excretion of phenothiazine in some water-soluble form, and of the reversible oxidation-reduction system thionol-leucothionol. These authors also stated, "In a personal communication, containing a report on the examination of a sample of the precipitate obtained from a phenothiazine urine, Mr. Wheeler of the Food Research Division, U. S. Department of Agriculture, has stated that the precipitate was largely phenothiazine plus a small amount of phenothiazone." However, it was impossible, at that time, to obtain additional evidence which was regarded as proof for the excretion of phenothiazone. This fact together with the statement in the literature (2) that phenothiazone and its leuco base are insoluble in water led to the conclusion that appreciable quantities of phenothiazone and its leuco base did not occur in the urine.

The fate of phenothiazine in sheep has been studied by Collier (3) who reported that the oxidation product was phenothiazone, excreted almost entirely in the form of the ethereal sulfate of leucophenothiazone. Subsequently an examination of the urine of sheep dosed with pheno-

Received for publication, October 11, 1941.

* Agricultural Chemical Research Division Contribution No. 43.

thiazine was also made by Lipson (4). In commenting on the report by DeEds, Eddy and Thomas, Lipson stated, "Their results, however, cannot be accepted as conclusive, as they were unable to isolate a crystalline product or derivative of thionol, and were forced to rely upon the method of oxidation-reduction potentials in order to characterize their product. As phenothiazone, another oxidation product of phenothiazine, will also give rise to a reversible oxidation-reduction system, this compound may have been present in the urine examined by these workers."

This statement implies that determination of the potential of a reversible oxidation-reduction system and comparison of the potential with that of a system of known identity are not reliable criteria for establishing the identity of the system in question. The further implication seems to be that the method of potential measurement would not have permitted the differentiation of the two systems phenothiazone-leucophenothiazone and thionol-leucothionol.

Because the measurement of oxidation-reduction potentials, where such measurements are possible, is not an orthodox procedure for identification purposes, justification for its use merits some discussion. The usual procedure of identification by melting point and mixed melting determinations represents an identical behavior of a known and unknown system at one point. The plotting of the oxidation-reduction potential of a known system, having a ratio of reductant to oxidant equal to 1, against variations in pH, and the superposition on the resultant curve of potential values similarly obtained on an unknown system represents an identical behavior of the two systems at many points. Since the changes in slope of the curve at certain pH values are due to the presence of ionizable groups, the chances that superimposable curves represent different compounds must be quite remote. The possibility of confusing the system thionol-leucothionol having two hydroxyl groups in the leuco form with the more positive system phenothiazone-leucophenothiazone, having one hydroxyl group in the leuco form, is negligible. Evidence that the phenothiazone-leucophenothiazone system is excreted in the urine of rats, rabbits and man, and is more positive than the thionol-leucothionol system will be submitted below.

The following quotation from the paper by DeEds, Eddy and Thomas (1) contains the evidence, at that time not recognized, that phenothiazone and particularly its leuco base occur in the urine of rats, rabbits and humans receiving phenothiazine: "The combined dilute alcohol solutions, after removal of the phenothiazine, were found to contain a considerable amount of the leuco base of the red dye, as indicated by results of aeration, addition of hydrogen peroxide, alkalization, or oxidation facilitated by passage through a filter paper, all of which yielded a deep red solution, extractable with chloroform, as already described. Extraction of the

combined dilute alcohol solutions with ether in five-foot liquid extractors of about one liter capacity yielded a straw-colored ether extract. After spontaneous evaporation of the ether, the residue was extracted with boiling carbon tetrachloride and filtered while hot. The filtered carbon tetrachloride solution was chilled in an ice bath and evaporated to a small volume under a current of air. Fine needle crystals of an almost colorless product separated out, and were sucked dry on a micro suction filter. The product was dried in an Abderhalden dryer, and the melting point determined. The nearly colorless crystals slowly underwent spontaneous conversion to a dark red powder. The melting point of the crystalline product was found to be 172 to 174° C."

Before submitting proof that the crystalline product melting at 172–174° C is leucophenothiazone an error in the report by DeEds, Eddy and Thomas (1) should be pointed out. It was concluded that the product was probably the leuco base of thionol. If the potential of the system represented by the crystalline product melting at 172–174° C and its oxidant had been studied this error would not have been made. Likewise, if a sample of pure phenothiazone had been available at that time, a melting point determination of its leuco base would have been made and the error also avoided in that manner. Attempts to prepare phenothiazone at that time and for that purpose were unsuccessful.

Following the report by Collier, a sample of phenothiazone was obtained from that author, and additional quantities were prepared by the method of Pummerer and Gassner (5). Each of these products was examined as follows: The phenothiazone was dissolved in a small volume of 95 per cent ethyl alcohol, distilled water was added and the mixture brought to a boil. A sufficient quantity of powdered sodium hydrosulfite was then added to the concentrated hot solution of phenothiazone to reduce it completely to the leuco base. Clouding of the hot solution occurred due to the lower solubility of leucophenothiazone and to small amounts of free sulfur. The mixture was then cooled under tap water whereupon a considerable amount of a fine white crystalline precipitate formed. The precipitate was removed by centrifuging. This precipitate was dissolved in hot carbon tetrachloride and the hot solution filtered. The carbon tetrachloride solution was placed in an ice bath and evaporated to a small volume under a current of air. Minute needle crystals of leucophenothiazone separated out. The crystals were nearly colorless, but en masse had a faint purple tinge, which may have been due to slight surface oxidation. On standing exposed to air for several hours the product darkened, a reaction which was greatly accelerated by ammonia gas. The leuco base prepared from the material furnished by Collier and that prepared in our laboratory had a melting point of 172–173° C, and the mixed melting point remained unchanged.

Samples of the product isolated from rat, rabbit and human urine as described by DeEds, Eddy and Thomas were again examined and melting point determinations made. The value of 172–173° C was obtained and remained unchanged when mixed with an equal weight of synthetic leucophenothiazone. Therefore, the product obtained from rat, rabbit and human urine, melting at 172–173° C and previously regarded as the leuco base of thionol, was undoubtedly leucophenothiazone.

As far as we know this is the first time that the melting point of leucophenothiazone has been reported. Attempts to isolate crystals of leucothionol by a similar procedure and determine the melting point have failed. The crystals have been obtained but the spontaneous oxidation of leucothionol proceeds so rapidly that melting point determinations have not been feasible. It is entirely possible that melting point determinations could be made if all operations were carried out in an atmosphere of nitrogen.

The fact that leucophenothiazone can be prepared, isolated, and the melting point determined in the presence of air whereas the same cannot be accomplished with leucothionol shows that the reversible system phenothiazone-leucophenothiazone has a potential appreciably closer to the oxygen electrode than that of thionol-leucothionol. Therefore, in a urine containing both these oxidation-reduction systems, the usual reducing properties of urine would keep the phenothiazone system in the leuco form for a longer period of time than would be the case with the thionol system.

After examining the various fractions prepared from the urine of sheep dosed with phenothiazine, Lipson (4) stated that Fraction 2 (b) yielded, after three recrystallizations from water, a red compound melting at 161–162° C, and was shown to be phenothiazone. Lipson further states, "a very small portion of Fraction 2 (b) was insoluble in boiling water, and could be recrystallized from aqueous alcohol to give a yellow solid which melted indefinitely at 170° C. This was probably some phenothiazine which had not been precipitated by acid in Fraction 1." However, since the melting point of leucophenothiazone is 172–173° C, there is a reasonable probability that the compound described by Lipson may have been leucophenothiazone.

SUMMARY

1. Evidence has been presented to show that the reversible oxidation-reduction system phenothiazone-leucophenothiazone, as well as thionol-leucothionol, occurs in the urines of rats, rabbits and humans receiving phenothiazine.
2. The validity of potentiometric identification of a reversible oxidation-reduction system has been discussed.

3. The melting point of leucophenothiazone has been shown to be 172–173° C. Samples of leucophenothiazone isolated from rat, rabbit and human urines were identified by their melting points, namely 172–173° C, and by their mixed melting points with synthetic leucophenothiazone.

4. Leucothionol oxidizes so rapidly that it has been impracticable to obtain its melting point.

5. It has been suggested that a compound isolated from the urine of a phenothiazine-dosed sheep by another worker was leucophenothiazone and not phenothiazine.

BIBLIOGRAPHY

- (1) DE EDS, F., EDDY, C. W. AND THOMAS, J. O. 1938 Studies on phenothiazine: V. Fate of phenothiazine in the body. *J. Pharmacol. and Exper. Therap.* **64**: 250.
- (2) BEILSTEIN 1896 Zweiter Band, Dritte Auflage, p. 811.
- (3) COLLIER, H. B. 1940 The fate of phenothiazine in the sheep. *Canad. J. Res.* **18D**: 272.
- (4) LIPSON, M. 1940 An examination of the urine of sheep dosed with phenothiazine. *J. Exper. Biol. and Med. Sc.* **18**: 269.
- (5) PUMMERER, R. AND GASSNER, S. 1913 Über die Desmotropie o- und p-chinoider Salze in der Thiazinreihe. *Berl. Deutsche Chem. Gesellsch.* **46**: 2310.

PHYSIOLOGY OF A BACTERIA-FREE CULTURE OF *TRICHOMONAS VAGINALIS*. IV. EFFECT OF HYDROGEN ION CONCENTRATION AND OXYGEN TENSION ON POPULATION¹

GARTH JOHNSON²

Departments of Obstetrics and Gynecology, and Zoölogy, State University of Iowa,
Iowa City, Iowa

PART I

EFFECT OF HYDROGEN ION CONCENTRATION ON POPULATION

No record of previous investigations of the physiology of *Trichomonas vaginalis* in bacteria-free culture has been found in the literature. Numerous workers have cultivated the organism in the presence of bacteria, and its response to the hydrogen ion concentration of various media has been recorded. Bland et al (1932) reported successful culture in media which shifted from pH 6 to pH 8 during the culture period "without any very noticeable effect upon the growth of the flagellates." Lynch (1922) maintained cultures in several media which "are slightly alkaline and become more so." Andrews (1929) used adjusted media varying from pH 7 to pH 8 and found an optimum growth at pH 7.6. Davis (1929) obtained successful culture "provided the pH was similar to that of human blood." It is difficult to determine to what extent these findings were affected by the bacterial flora. Differences in the culture medium used as well as in the nature and concentration of the bacteria present undoubtedly affect the rate of growth.

Other trichomonads have been investigated in bacteria-free culture. In a study of *Trichomonas foetus* Cailleau (1937) found an optimum pH between 7.0 and 7.6 with survival between pH 5.5 and 8.5. Riedmüller (1936) reported an optimum pH range of pH 6.5 to 7.5 for the same organism and Morisita (1939) found the optimum between pH 6.6 and 7.8 with survival between pH 5.6 and 8.4. Cailleau (1937) cultivated *Trichomonas columbae* in media ranging from pH 6.6 to 7.6 and found that the addition of calcium carbonate prolonged the life of the cultures.

The experiments reported in this paper were designed to determine the extremes of hydrogen-ion concentration which would support growth

Received for publication, October 14, 1941.

¹ This paper represents a dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Zoology of the State University of Iowa, June, 1941.

² The author is indebted to Doctors T. L. Jahn, E. D. Plass, and Ray E. Trussell for their constant encouragement and aid in the course of this study.

of the organism in the absence of bacteria and to discover the optimum pH for a medium which would maintain repeated subculture.

MATERIALS AND METHODS

The culture medium consisted of Difco liver infusion agar slants (3 ml) overlaid with 6 ml of modified Ringer's solution containing 5% of human serum. The Ringer's solution contained 0.6% NaCl and 0.01% NaHCO_3 , KCl, and CaCl_2 . The pH of both the agar slants and the fluid medium was adjusted with normal HCl or normal NaOH, after which they were buffered with 0.25% sodium phosphate. This concentration of buffer supported the maximum population with only minor changes in pH during the first four days of culture and was chosen after observation of cultures buffered with 0.1, 0.25, 0.50, and 0.87% sodium phosphate. The organisms were cultured in 13×120 mm tubes, plugged with cotton and incubated at $37.5 \pm 1^\circ \text{C}$. All manipulations of the organisms were made with cotton-stoppered pipettes, sterilized by hot air and controlled by mouth suction. The pH determinations were made with a glass electrode and a Cameron pH meter. Before each series of determinations the electrode was checked against buffers standardized at pH 4.0 and 7.38. One ml of culture fluid was withdrawn into a clean test tube and diluted with 2 ml of distilled water. After thorough agitation this fluid was transferred to the cup of the pH meter. The error due to discharge of carbon dioxide from the fluid was probably negligible, since the production of carbon dioxide was not demonstrable. In each case the pH of tube number 1 in each group was checked at each period of examination with that of the corresponding uninoculated control tube.

The populations were determined with a hemocytometer after 2, 4, and 6 days of incubation. Each count included 10 large squares having a total volume of 1.0 cu mm.

Aseptic precautions were observed throughout the experiments and at the conclusion of each series the cultures were checked for bacterial contamination by plating them on blood agar or chocolate agar. Less than one per cent of the cultures were found to be contaminated, and the data pertaining to these specimens are not included below.

The stock cultures originated from the bacteria-free culture isolated by Ray E. Trussell (1940) and were checked for bacterial contamination as reported by Trussell and Plass (1940).

An inoculum consisting of 0.2 ml of a uniform suspension of organisms combined from several four-day-old stock cultures was used in each experiment. Six tubes were inoculated at each of sixteen different reactions between pH 7.8 and 5.1. One uninoculated tube was maintained at each pH as a control. All cultures in each series were inoculated from the same suspension of organisms. It was therefore unnecessary to con-

sider the initial population as a variable factor. Series I and Series II are duplicates. The cultures in Series I were also subcultured on the fourth day.

RESULTS

Maximum populations in both series and in the subcultures were found on the acid side between pH 5.1 and pH 6.0. The growth optimum shifted with the time of incubation. In Series I, the optimum at 48 hours was between 5.25 and 5.4, at 96 hours between 5.5 and 6.0, while at 144 hours it dropped again to a range of pH 5.15 to 5.5 (Fig. 1). The

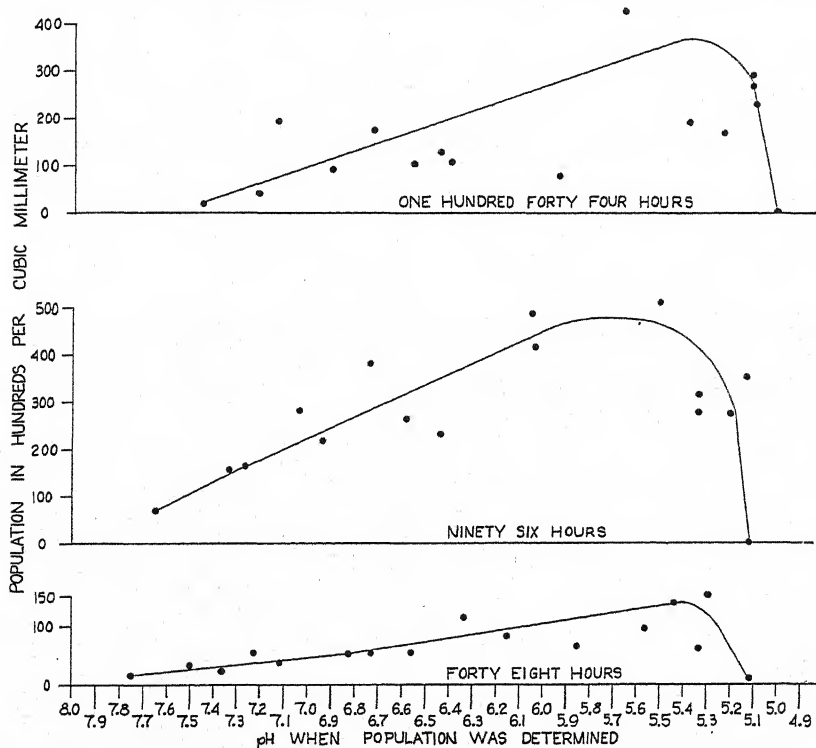


FIG. 1. Series I. Population plotted against pH. Curves fitted free hand.

optima of Series II approximated those of Series I as follows: At 48 hours, pH 5.45 to 5.55; at 96 hours pH 5.5 to 5.8, at 144 hours pH 5.15 to 5.55. Population values fell off sharply as the pH was reduced below the optimum, and multiplication ceased below pH 5.0 (Fig. 1). Broader ranges of pH were tolerated on the alkaline side of the optima with multiplication practically stopped between pH 7.55 and 7.75 (Fig. 1). Subculture was unsuccessful above pH 7.55.

Minor fluctuations occurred in the pH of both the uninoculated controls and the experimental cultures. Comparison of the pH of experi-

mental and uninoculated control tubes after the same intervals of incubation indicates that these changes in pH do not have a significant bearing upon the conclusions to be drawn from the data.

DISCUSSION

The correlation between these observations of a slightly acid optimum pH in bacteria-free cultures of *Trichomonas vaginalis* and the pH of the vaginal secretion in clinical cases of human trichomonas vaginitis has already been pointed out by Trussell and Plass (1940). Trussell and MacDougal (1940) employed a glass electrode technique in an investigation of the relation of vaginal acidity to various types of vaginal flora. In 18 out of 23 cases where vaginal trichomonads were observed they found an average vaginal pH of 5.58 with a range from pH 4.55 to 6.38. It is well known that introduction of acid solutions, such as dilute vinegar or acetic acid, are helpful in the treatment of trichomonas vaginitis. The very narrow range which has been observed to separate the optimum from the lower limit for multiplication on the acid side possibly demonstrates the mechanism behind this clinical observation.

There is apparently no record of other attempts to culture *Trichomonas vaginalis* in media adjusted to reactions well down in the pH scale and buffered to hold the reaction relatively constant. This in itself would account for the reports by other workers of an alkaline optimum. Bland et al (1932) started cultures at pH 6.8 and found that the pH shifted to the alkaline side in both experimental and control tubes. If they had started their cultures at a more acid reaction different conclusions might have been drawn. In addition to the possible effect of the presence of bacteria in cultures studied by earlier workers, one other factor should be considered. In any case where the pH of the culture medium was initially high and was allowed to drift down the scale and across an acid optimum the length of time elapsing before an acid limit would be reached would inevitably be longer with a higher total population than if the initial pH were lower. Thus if only the original pH were considered the optimum pH might appear to be at a higher level.

SUMMARY

1. Under the conditions of these experiments *Trichomonas vaginalis* exhibits a maximum population in an acid medium between pH 5.5 and 6.0.
2. Multiplication is limited or stopped by reactions below pH 5.0 and above pH 7.55.
3. There appears to be a correlation between these results and the conditions in the human vagina in cases of natural infection.
4. The use of acid solutions in the treatment of trichomonas vaginitis

is in harmony with the limited range between the optimum pH and the acid limit of multiplication.

PART II

EFFECT OF OXYGEN TENSION ON POPULATION

Riedmuller (1936) found the growth of *Trichomonas foetus* in bacteria-free culture was stimulated by anaerobiosis. He was also able to demonstrate an aerobic utilization of oxygen by the Warburg technique. It is difficult to interpret the finding of oxygen consumption in association with a stimulation of population under anaerobic conditions unless Riedmuller's classification of *Trichomonas foetus* as a facultative anaerobe is accepted.

Aerobic culture of *Trichomonas vaginalis* in this laboratory has always involved the incorporation of an infusion of beef liver in the culture medium. It has been demonstrated (unpublished data) that a reduction in the amount of liver infusion lowers the population level. In a study of carbohydrate utilization, Trussell and Johnson (1941) used a basic medium lacking liver infusion and found that it was necessary to establish anaerobic conditions by the addition of sodium thioglycollate before there was a measurable effect of carbohydrate on population. This stimulating effect of reduced oxygen tension is easily demonstrated by comparing the growth of stock cultures in an anaerobic jar with that in air.

In an attempt to throw further light on the anaerobic character of the organism a quantitative study of population at different oxygen tensions was undertaken.

MATERIALS AND METHODS

Experience gained in the early phases of this study led to alterations in the formula of the culture medium. In a fluid medium of low viscosity the population of trichomonads becomes much denser at the bottom of the culture. A more homogeneous culture medium and especially one in which the organisms will remain in suspension was prepared as follows:

Bacto liver infusion	80.0 ml ³
Bacto heart infusion powder	25.6 gm
Bacto agar	0.4 gm
Distilled water	720.0 ml
Normal HCl	6.0 ml

This mixture was heated to boiling, tubed in 8.0 ml volumes and autoclaved. After cooling, 2.0 ml of sterile undiluted human serum adjusted

³ Liver infusion was prepared by adding 75 gm Bacto dehydrated liver to 1 liter of distilled water, heating at 50° C for one hour and filtering after raising the temperature to 80° C.

to pH 6 were added to each tube. The pH of the final mixture ranged from 6.1 to 6.2. Choice of this medium was based upon preliminary investigations which revealed that a mixture containing 10% liver infusion and 20% serum plus the other ingredients listed would support a relatively dense population without significant shift in pH. Variations in population between cultures subjected to the same experimental conditions were also greatly reduced.

The inoculum was prepared by combining 3.0 ml of fluid from each of two stock cultures and adding 4.0 ml of the experimental medium without serum. This gave a suspension in which the organisms showed little tendency to settle either in the tube or in the pipette. The inoculations were made by transferring 0.2 ml of the fluid from this suspension of organisms to the mid level of each culture tube and stirring the culture thoroughly with the tip of the pipette.

In Series III the cultures were maintained under complete anaerobiosis, 100 mm oxygen tension and 500 mm oxygen tension. A fourth set of cultures was kept in an open rack as controls. Series IV was a duplication of Series III.

In Series V the culture conditions included complete anaerobiosis, 100 mm oxygen tension, pure oxygen, and a set of controls kept as before under atmospheric conditions. Series VI duplicated Series V.

Anaerobiosis was established in McIntosh and Fildes jars, improved model. The procedure followed was that recommended by the manufacturer. No evidence of impurity due to carbon dioxide was obtained when the hydrogen which was employed was allowed to bubble through filtered lime water for ten minutes.

The cultures were subjected to various oxygen tensions in McIntosh and Fildes jars by blowing a stream of hydrogen through the jar for five minutes, evacuating it to the desired negative pressure and then introducing oxygen to bring the manometer reading to zero. No evidence of carbon dioxide appeared when the oxygen which was employed was bubbled through filtered lime water for ten minutes. The highest concentration of oxygen was obtained by running a vigorous stream of the gas through an aluminum jar for five minutes. This jar was sealed by a gasket and the lid was tightened by adjusting three screw clamps at the edges of the lid. To what extent the resulting atmosphere was contaminated by residual nitrogen and other gases already in the jar and in the tubes is not known. The data from these cultures are labeled "barometric oxygen" subject to the above reservations.

Ten tubes were run at each oxygen tension, making a total of forty tubes for each series. At each counting period the four sets of cultures were removed from the incubator at the same time and brought to atmospheric conditions and room temperature by removing the lids from the

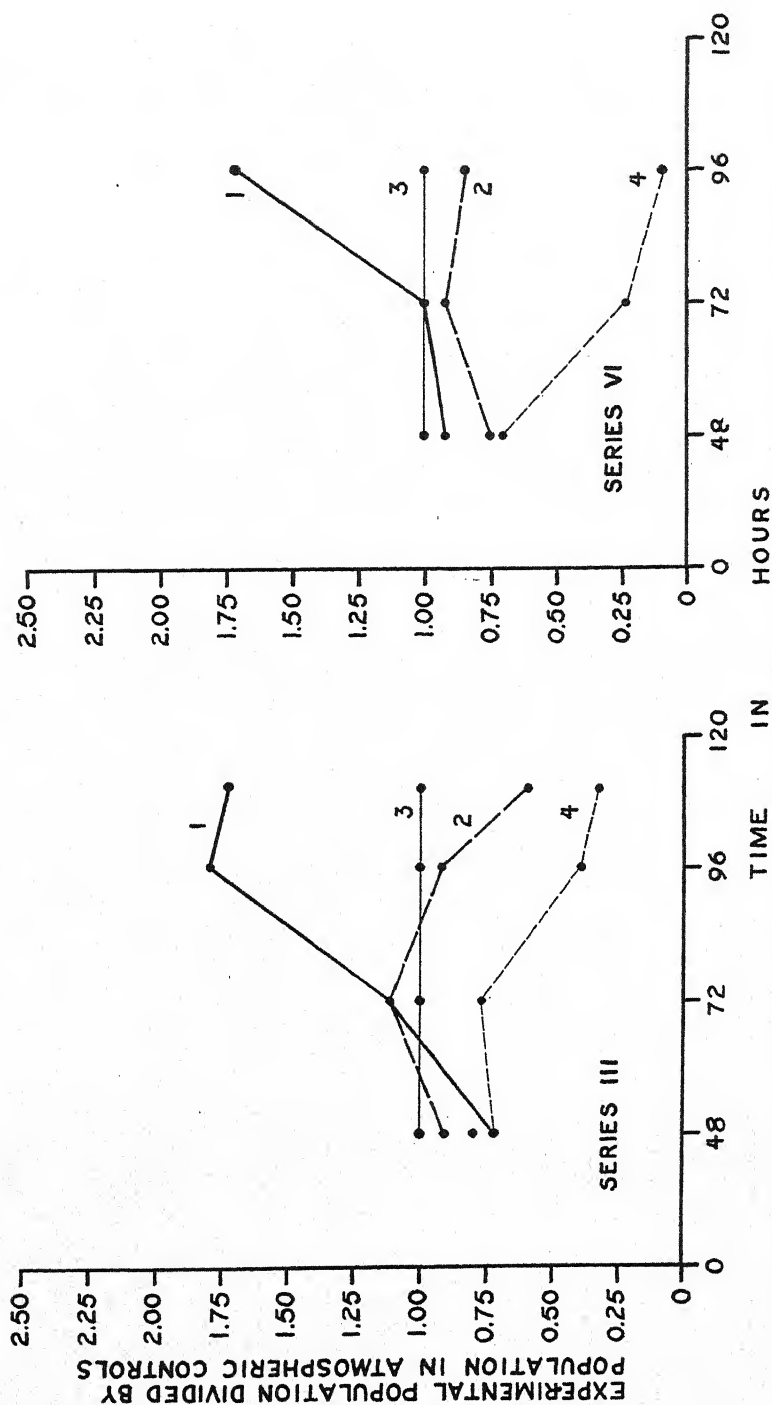


Fig. 2. Ratio experimental population over population in control cultures plotted against time. Curves as follows: Series III. 1—complete anaerobiosis, 2—100 mm oxygen tension, 3—controls, 4—500 mm oxygen tension. Series VI. Curves 1-3 as before, 4—barometric oxygen tension.

jars. It has been found that no multiplication of this bacteria-free culture occurs below 28°C (unpublished data). Therefore, it is assumed that the population values determined represent the result of multiplication while the cultures were subject to experimental atmospheres at the incubation temperature of 35°C . The latter temperature was adopted because the stock cultures had been maintained at this temperature for several months prior to the beginning of these experiments.

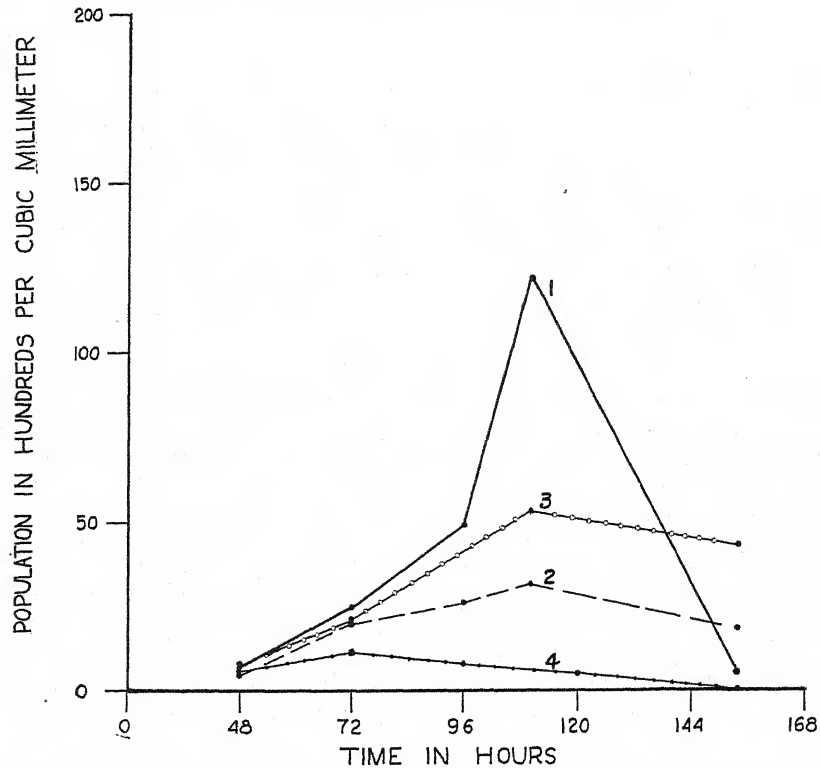


FIG. 3. Series IV. Population plotted against time. Curves: 1—complete anaerobiosis, 2—100 mm oxygen tension, 3—controls, 4—500 mm oxygen tension.

The pH values of the various cultures were determined with a glass electrode. In Series III and IV the reaction of tubes 1, 2, and 3 in each set was measured at the first three periods of observation. At the fourth and fifth periods it was determined for tubes 4, 5, and 6. A similar procedure was followed in Series V and VI except that it was unnecessary to make more than two successive determinations on any one culture since there were in all only four counting periods. To determine the error involved in diluting the culture fluid with two parts of distilled water, duplicate determinations on the diluted and undiluted culture fluid were made at the conclusion of Series III and IV. Populations were deter-

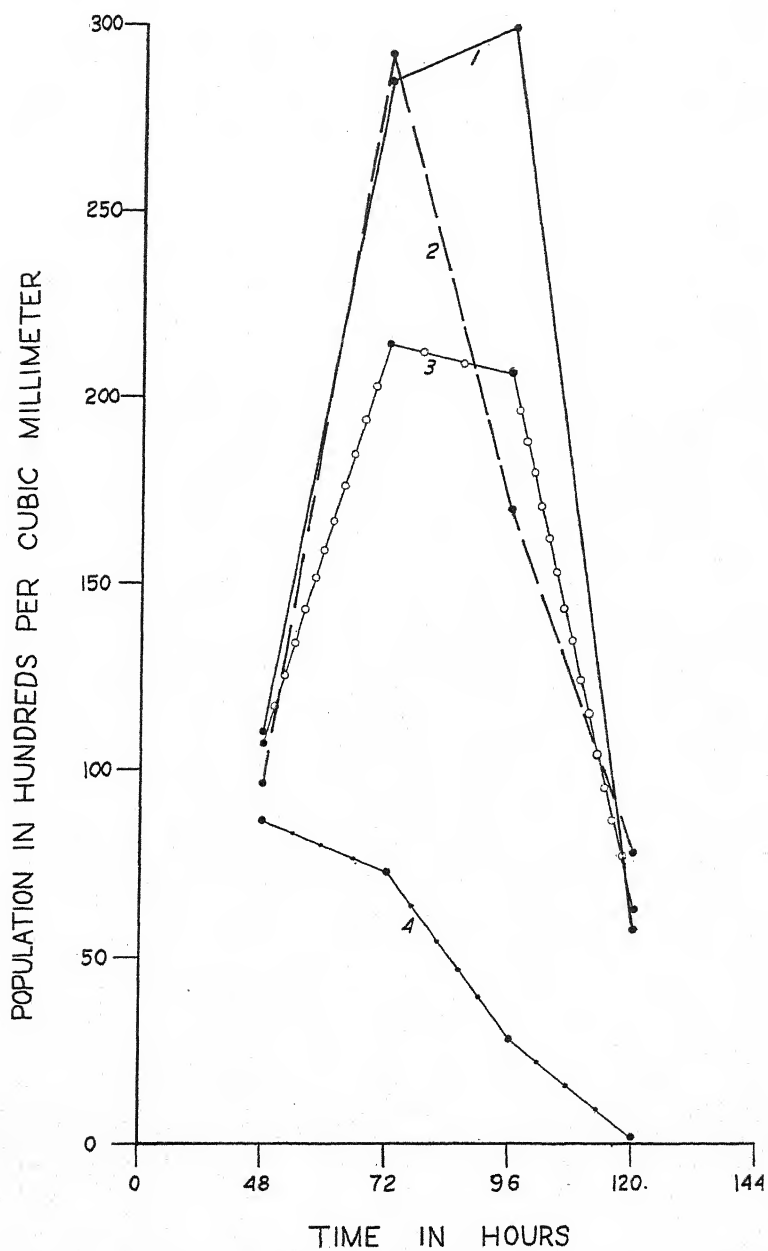


FIG. 4. Series VI. Population plotted against time. Curves: 1—complete anaerobiosis, 2—100 mm oxygen tension, 3—controls, 4—barometric oxygen tension.

mined with a hemacytometer. Following the first 48 hours of culture the measurements, unless otherwise indicated, were made at successive 24-hour intervals until a definite decline in population was observed.

Since the trichomonads produce only a faint clouding of this medium, gross contaminations were easily detected by direct observation. At the conclusion of each series all cultures were streaked on chocolate agar and the plates incubated aerobically in a moist jar for two days. A check for anaerobic contaminations was then made by keeping the same plates in a McIntosh and Fildes jar for two weeks. As a further check for anaerobic contaminations cultures 4, 5, and 6 in each set were inoculated into sodium thioglycollate medium containing glucose. No data are included from cultures shown to be contaminated. The contaminated cultures amounted to 4.5 per cent of the total and the data from these were excluded.

RESULTS

All tensions of oxygen employed in these experiments exerted some depression upon the population level. This fact is demonstrated by a comparison of the ratios of experimental populations to those of the atmospheric control cultures. In each case (Figs. 2, 3 and 4) the curve for cultures subjected to an atmosphere containing oxygen falls below that for cultures maintained under complete anaerobiosis. The depression of population by oxygen tensions of 150 mm Hg (controls) or less did not become significant until after 72 hours in any of the four series. The organisms did not appear to be sensitive to the difference of 50 mm oxygen tension between the control group (150 mm) and the cultures kept under 100 mm oxygen (curves 2 and 3, Figs. 3 and 4). No explanation is offered for the fact that in two out of four series the curve for 100 mm oxygen tension fell slightly below that of the controls. The depression of population in oxygen at barometric pressure was marked and continuous after the first 48 hours (curve 4, Fig. 4), whereas there was a slight rise in population under 500 mm oxygen between 48 and 72 hours (curve 4, Fig. 3). Complete anaerobiosis prolonged the period of population increase and constituted the optimum culture condition (curve 1, Figs. 3 and 4).

Very minor changes in pH occurred in the course of incubation and an average error of only 0.07 of one pH unit toward the alkaline side was introduced by diluting the culture fluid with two parts of distilled water.

DISCUSSION

Trichomonas vaginalis normally inhabits an environment in which it is probable that oxygen tension is low, particularly in the vaginal fornices. It is not therefore surprising to find that experimental populations rise to a maximum as oxygen tension approaches zero. Since it has been found

that this parasite is unable to utilize carbohydrates under aerobic conditions but can do so in the presence of a sulfhydryl compound like sodium thioglycollate (Trussell and Johnson, 1941), it seems probable that the -SH supplied by liver infusion may have contributed some measure of anaerobiosis in these experiments. Further investigation with a medium containing less liver infusion might reveal a more marked depression of population by oxygen. It was the purpose of this study to test the effect of oxygen tension when other conditions were optimum. The results make an investigation of the oxidation-reduction potentials in the culture fluid seem worthwhile.

SUMMARY

1. In these studies *Trichomonas vaginalis* has exhibited the characteristics of a facultative aerobe.
2. Oxygen exerts a marked depressing effect upon population.
3. Anaerobic conditions prolong the period of population increase and are optimum for the culture of the organism.

BIBLIOGRAPHY

- ANDREWS, M. N. 1929 Observations on *Trichomonas vaginalis* Donné. J. Trop. Med. 32: 237-240.
- BLAND, P. B., GOLDSTEIN, L., WENRICH, D. H. AND WEINER, E. 1932 Studies on the biology of *Trichomonas vaginalis*. Am. J. Hyg. 16: 492-512.
- CAILLEAU, R. 1937 La nutrition des flagellés tetramitidés. Les sterols, facteurs de croissance pour les trichomonades. Ann. Inst. Pasteur 59: 137-172.
- DAVIS, C. H. 1929 *Trichomonas vaginalis* Donné. Second report of experimental and clinical observations. Am. J. Obst. and Gynec. 18: 575-579.
- JOHNSON, GARTH 1940a Preliminary studies of a pure culture of *Trichomonas vaginalis*. Anat. Rec. 78 Suppl: 179.
- 1940b Physiology of a pure culture of *Trichomonas vaginalis*. I: Population in relation to pH. Proc. Soc. Exper. Biol. and Med. 45: 567-569.
- LYNCH, K. M. 1922 Cultivation of *Trichomonas* from the human mouth, vagina, and urine. Am. J. Trop. Med. 2: 531-538.
- MORISITA, T. 1939 Studies on the trichomonad parasitic in the reproductive organs of cattle. Japan. J. Exper. Med. 17: 1-63.
- RIEDMULLER, L. 1936 Beitrag zum kulturellen Verhalten von *Trichomonas foetus*. Centr. Bakt. (Orig.) 137: 428-433.
- TRUSSELL, R. E. 1940 Experimental and clinical trichomonas vaginitis. J. Iowa Med. Soc. 30: 66-70.
- TRUSSELL, R. E. AND JOHNSON, GARTH 1941 Physiology of a pure culture of *Trichomonas vaginalis*. III: Fermentation of carbohydrates and related compounds. Proc. Soc. Exper. Biol. and Med. 47: 176-178.
- TRUSSELL, R. E. AND PLASS, E. D. 1940 The pathogenicity and physiology of a pure culture of *Trichomonas vaginalis*. Am. J. Obst. and Gynec. 40: 883-890.
- TRUSSELL, R. E. AND MACDOUGAL, R. F. 1940 Vaginal acidity (in vivo glass electrode measurements) in late pregnancy and its relation to the vaginal flora. Am. J. Obst. and Gynec. 39: 77-81.

CRENOSOMA ZEDERI N. SP. (NEMATODA: METASTRONGYLOIDEA), A NEW LUNGWORM FROM THE SKUNK (*MEPHITIS NIGRA*)

FRANS C. GOBLE

Wildlife Research Center, N. Y. State Conservation Dept., Delmar, New York

During a recent investigation on the parasites of fur-bearers in New York State, this apparently new species of lungworm was found in the lower bronchi of two skunks, wild-trapped in Albany County. It is herein described and named in honor of J. G. H. Zeder, who described and named the first species of the genus *Crenosoma* in 1800.

Crenosoma zederi n. sp.

(Figs. 1-4)

Male: 1 specimen, 6 mm long, 330 micra maximum width, 100 micra wide just anterior to bursa. Cuticular folds present over body back as far as anterior ends of spicules. Folds 60 micra apart at base esophagus, increasing to 80 micra in middle of body, decreasing near end of folded region. Parallel longitudinal ridges over entire surface of cuticle. Esophagus 395 micra long, 58 micra in maximum width, near posterior end. Excretory pore opens 80 micra from anterior end. Bursa 200 micra wide, notched at ends of latero-ventral, postero-lateral and dorsal rays. Distinct ventral lobe present, notched at edge medioventrally. Rays stout. Ventrals divided at tips. Externo-laterals separate from medio- and postero-laterals which lie close to each other and to externo-dorsal, the last three rays being parallel and directed posteriorly at 45° angle from axis of worm. Dorsal stout and simple, 45 micra long and 28 micra wide. Spicules equal, lying close to each other, bent slightly ventrally at both ends. They are 265 micra long, 8 micra in maximum width, near anterior ends. Distal ends not distinctly swollen. Unpaired accessory piece 100 micra long, divided into coarsely granular anterior portion 40 micra long, half-moon shaped in lateral view, and smooth, narrow posterior portion 60 micra long, which tapers posteriorly and ends in sharp point.

Female: 5 specimens, measuring 24, 24, 25, 25, and 26 mm long, 480-560 micra in maximum width, 240 micra wide at base esophagus. Cuticular folds present over entire body; 80 micra apart at base esophagus, space increasing to 120 micra at vulva and decreasing to about 60 micra at anus. Longitudinal ridges as in male. Esophagus 480-560 micra long, 90-100 micra maximum width. Excretory pore 180-190 micra from anterior end. Vulva 10-10.5 mm from anterior end. Anus 160-170 micra from posterior end. Two lateral phasmids, not extending through surface of cuticle, 40-50 micra from tip of tail. Larvae in utero 17-20 micra in diameter, possess lateral alate swellings as described in *C. potos*.

Host: *Mephitis nigra* (Peale and Beauvois, 1796).

Locality: Delmar, Albany County, New York.

Type specimens: U. S. N. M. Helm. Coll. No. 45005.

The male of *Crenosoma zederi* can be distinguished from those of *C. striatum* and *C. taiga* by its lack of an obvious dorsal branch on the spicule. It differs from males of *C. vulpis* and *C. mephitidis* in the shape of the gubernaculum, stoutness of the bursal rays and in its lack of lateral

Received for publication, November 16, 1941.

projections at the tip of the dorsal ray. It differs from those of *C. potos* in the shape of the gubernaculum and stoutness of rays and can be distinguished from the males of all described species by the presence of cuticular folds over the body as far back as the anterior ends of the spicules. In describing *C. potos* Buckley (1930) mentions that the folds are represented by regularly spaced transverse smooth bands of cuticle on the posterior part of the males where the actual folding has ceased and that the longitudinal "ridges are present over the whole surface of the body, except immediately posterior to each fold, which is smooth for a short distance." These conditions are not observed in *C. zederi*, in which there are no interruptions of the longitudinal ridges whatsoever.

The females of *Crenosoma zederi* can be distinguished from those of *C. striatum* by the lack of a cuticular swelling at the vulva, and from all other species by the presence of cuticular folds over the entire body. Though the margins of the folds, as in other species of *Crenosoma*, appear to be spinous on superficial examination, with higher magnifications it is readily observed that this illusion results from the folding of the longitudinal ridges and that no actual spinous structure exists. Certain measurements and size relationships in both sexes of *C. zederi* differ from those of other species of the genus, but since there is some variability within each species and some overlapping in the measurements between the species, it seems advisable to minimize the quantitative and emphasize the qualitative characters.

In reviewing the characters of the described species of *Crenosoma*, I have noted some discrepancies on which comment seems desirable. Hobmaier (1941a) in describing *C. mephitidis* referred to Buckley's table of measurements of the known species of *Crenosoma* and remarked that *C. mephitidis* had certain resemblances to *C. vulpis*. In a later paper (1941b) he again emphasizes that *C. mephitidis* is morphologically similar to *C. vulpis* and that they might be confused. He (1941a) mentions as points of differentiation the presence of cervical glands in *C. mephitidis*, a size difference, especially in females, and the presence of a longer tail in females of *C. mephitidis*.

Buckley quotes part of Dujardin's (1845) description pointing to the presence of cervical glands in *C. vulpis* and Hobmaier himself (1941a) suggests that they may be a generic character. In view of the number of strongylins in which excretory glands have been reported it seems likely that their presence is a character common to a rather large group. At any rate they cannot be used to separate *C. mephitidis* from *C. vulpis*. Since worms of the same species may be expected to show differences in size when found in different hosts, it seems inadvisable to consider the difference between 15 mm length in females of *C. vulpis* and 18 mm in those of *C. mephitidis* a specific character.

Hobmaier's statement that the tail is longer in *C. mephitis* is based on comparison of his measurements with those given by Buckley for *C. vulpis*. Buckley's table was compiled from measurements of Skrjabin and Petrow (1928) who gave, among other measurements for *C. vulpis*, the following: Accessory piece, 0.550 mm, tail, 0.870 mm. These measurements obviously are erroneous and undoubtedly represent typographical errors. Buckley gave these measurements in his table, following each with parenthetical question marks. He noted beneath the table that the figures queried probably should read "0.055 mm." and "0.087 mm.," apparently assuming that the error was simply in the position of the decimal point. No scale is given on the drawings of Skrjabin and Petrow, but checking the drawings against considerable material of *C. vulpis* in my collection and against measurements of organs given correctly in their text, it is evident that their drawings are correct and the correct measurement for the accessory piece is 125–135 micra and for the tail length 130–160 micra. Hobmaier gives the tail length of *C. mephitis* as 140–150 micra.

BIBLIOGRAPHY

- BUCKLEY, J. J. C. 1930 On a lungworm *Crenosoma potos* n. sp. from the kinkajou, *Potos flavus* (Schreber). J. Helm. 8: 229–238.
- HOBMAIER, M. 1941a Description and extramammalian life of *Crenosoma mephitis* n. sp. (Nematoda) in skunks. J. Parasitol. 27: 229–232.
- 1941b Newer aspects of the lungworm (*Crenosoma*) in foxes. Am. J. Vet. Res. 2: 352–354.
- SKRJABIN, K. I. AND PETROW, A. M. 1928 A description of the genus *Crenosoma* Molin, 1861 (Metastrongylidae, Nematoda). Parasitology 20: 329–335.

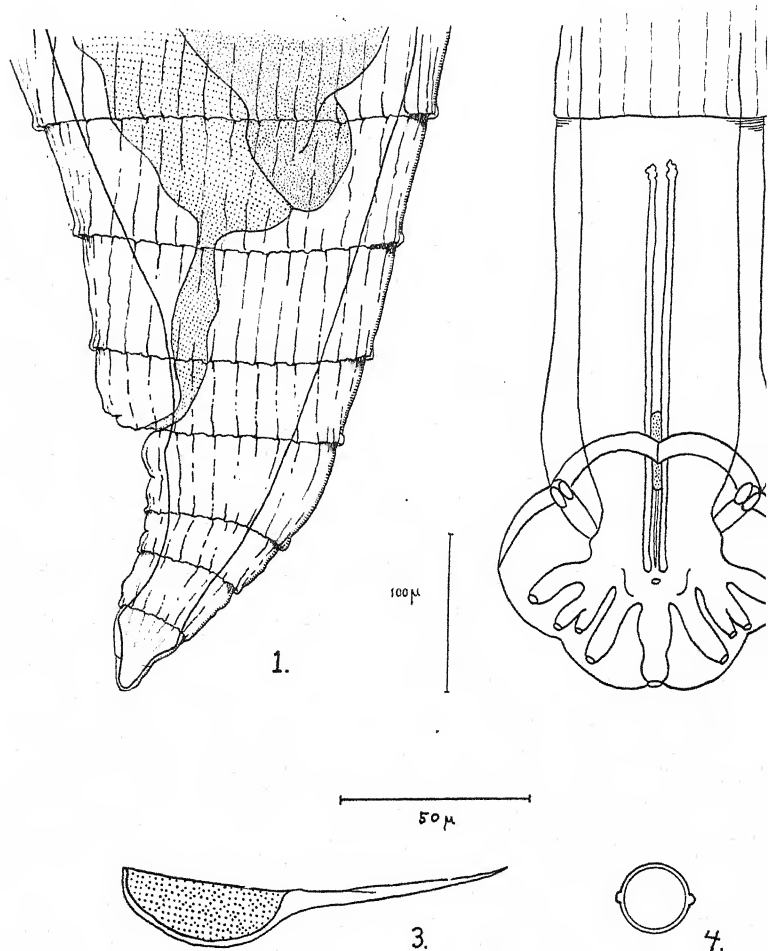


PLATE I

Crenosoma sederi n. sp.

FIG. 1. Posterior end of female, latero-ventral view.

FIG. 2. Posterior end of male, ventral view.

FIG. 3. Unpaired accessory structure, lateral view.

FIG. 4. First stage larvae, optical cross section.

(The vertical scale applies to Figs. 1 and 2, the horizontal scale to Fig. 3 and 4.)

A NEW SPECIES OF THE ACANTHOCEPHALAN
GENUS *FILICOLLIS*

MARY LOUISE PERRY*

Department of Zoölogy, University of California at Berkeley

In the late autumn of 1938 and the winter of 1940, I noticed dead and dying surf scoters (*Melanitta perspicillata*) and white-winged scoters (*M. deglandi*), all females, on the shore of Carmel Bay, Monterey Co., Calif. In 1938, a number of acanthocephalan worms were found in the intestines of a surf scoter and two white-winged scoters. On Feb. 24, 1940, I noted five dead surf scoters and one dead white-winged scoter. Beach scavengers had eaten the intestines of all but two surf scoters, which were parasitized by these acanthocephalans. The worms attached themselves by imbedding their hook-covered proboscides in the intestinal wall, their bodies hanging loose in the lumen (cf. Fig. A). In addition to these worms, four minute gizzard worms (nematodes), and one large tapeworm 250 mm posterior to the gizzard were found in the surf scoter taken Feb. 24.

2. All of the intestines observed were heavily infected. I examined in detail the alimentary tract of one surf scoter from the proventriculus posteriorly. Infection with the acanthocephalans began 250 mm posterior to the gizzard and continued to the anus, although the ceca were not infected. Worms became progressively smaller at the approach of the large intestine, although there were a few small worms scattered among the large ones throughout the small intestine. Infection was heaviest at the anterior end. According to my computations, there were 1,482 worms in the 28,000 sq mm of intestine.

The heavy infection of the scoters probably caused their death, or at least made them very susceptible to secondary infection. They had no subcutaneous layer of fat, and were too weak to cope with the waves or attempt to escape from approaching people or dogs.

Most of the worms mounted were fixed in hot 70% alcohol, stained in alum cochineal or Grenacher's borax-carmine, and occasionally counterstained with indulin blue dye. They were cleared in creosote-xylol, and mounted in balsam. Embryos were always counterstained in indulin blue dye, and were mounted in glycerin jelly.

On the basis of the slides examined, I propose a new species of the

Received for publication, December 1, 1941.

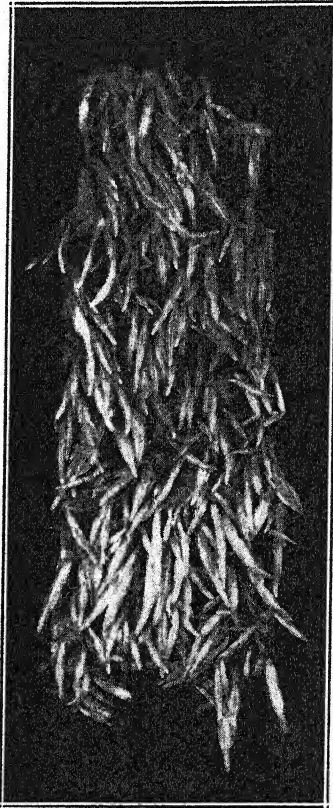
*I wish to acknowledge the assistance of Professor Harold Kirby (University of California, Berkeley), under whose guidance I carried on this research, and also that of Professor H. J. Van Cleave (University of Illinois, Urbana), who offered much helpful criticism on this paper.

genus *Filicollis*, which I name after Dr. Luther C. Altman, zoölogist, Lincoln High School, Seattle, Wash.

Filicollis altmani n. sp.

(Figs. 1-4; Text-Fig. A)

Body elongate, nearly cylindrical, with an even or uneven division of body surface by two strictures. Mature males 10.5 mm long (8.5-12); mature females 12.25 mm long (10.5-14). Diameter 1.67 mm (1.2-2.1). Neck long, slender, retractable,



TEXT-FIG. A. *Filicollis altmani* attached to the inner wall of the small intestine of a surf scoter. Photograph, natural size.

about 1.38 mm long (1.08-1.5); width at base 0.44 mm (0.3-0.55). Both sexes with minute body spines, confined to anterior part of body. Proboscis of both sexes characteristically oblatly spheroidal, 0.7 mm \times 0.8 mm (0.62-0.8 \times 0.5-0.96); occasionally spherical or ovoid; bearing 28 (25-30) longitudinal rows of hooks with 11 (9-12) hooks per longitudinal row. Basal hooks 0.056 mm long (0.05-0.07); apical hooks, 0.038 mm long (0.03-0.05); and other hooks 0.047 mm long (0.03-0.06). Proboscis receptacle double walled, 2.2 mm long (1.7-2.75). Lemnisci about 0.05 mm shorter than the receptacle. Male genitalia occupy about two-thirds of the length of the body cavity. Testes spherical or ovoid. Four cement glands, long and tubular. Hard shelled embryos 0.065 \times 0.026 mm (0.06-0.07 \times 0.022-0.03); ex-

clusive of the shells, 0.041×0.012 mm ($0.04-0.042 \times 0.01-0.014$). Embryos with three conspicuous shells; middle shell shape of ellipse with pointed ends, so showing slight tendency toward polar prolongation.

Type hosts: *Melanitta perspicillata* (Linnaeus) and *Melanitta deglandi* (Bonaparte) taken at Carmel Bay, Monterey Co., Calif. Nov. 11 and 18, and Dec. 4, 1938, and Feb. 24, 1940.

Habitat: Small and large intestine.

Holotype male (slide 3592.1 Van Cleave), allotype (slide 3592.2 Van Cleave), and paratypes at the Univ. of Ill. at Urbana. Paratypes at the Univ. of Calif. at Berkeley.

Filicollis altmani differs from *F. anatis* in the number of longitudinal rows of hooks on the proboscis, the number and shape of the cement glands, and the shape of the middle shell of the embryo. It differs from *F. sphaerocephalus* in the length of the lemnisci, number of cement glands, and the shape of the middle shell of the embryo.

EXPLANATION OF PLATE, p. 388

All figures drawn with the aid of a camera lucida.

FIG. 1. Holotype male of *Filicollis altmani*.

FIG. 2. Anterior end of paratype female.

FIG. 3. Middle section of proboscis of holotype male.

FIG. 4. Embryo from surface of mucosa of small intestine.

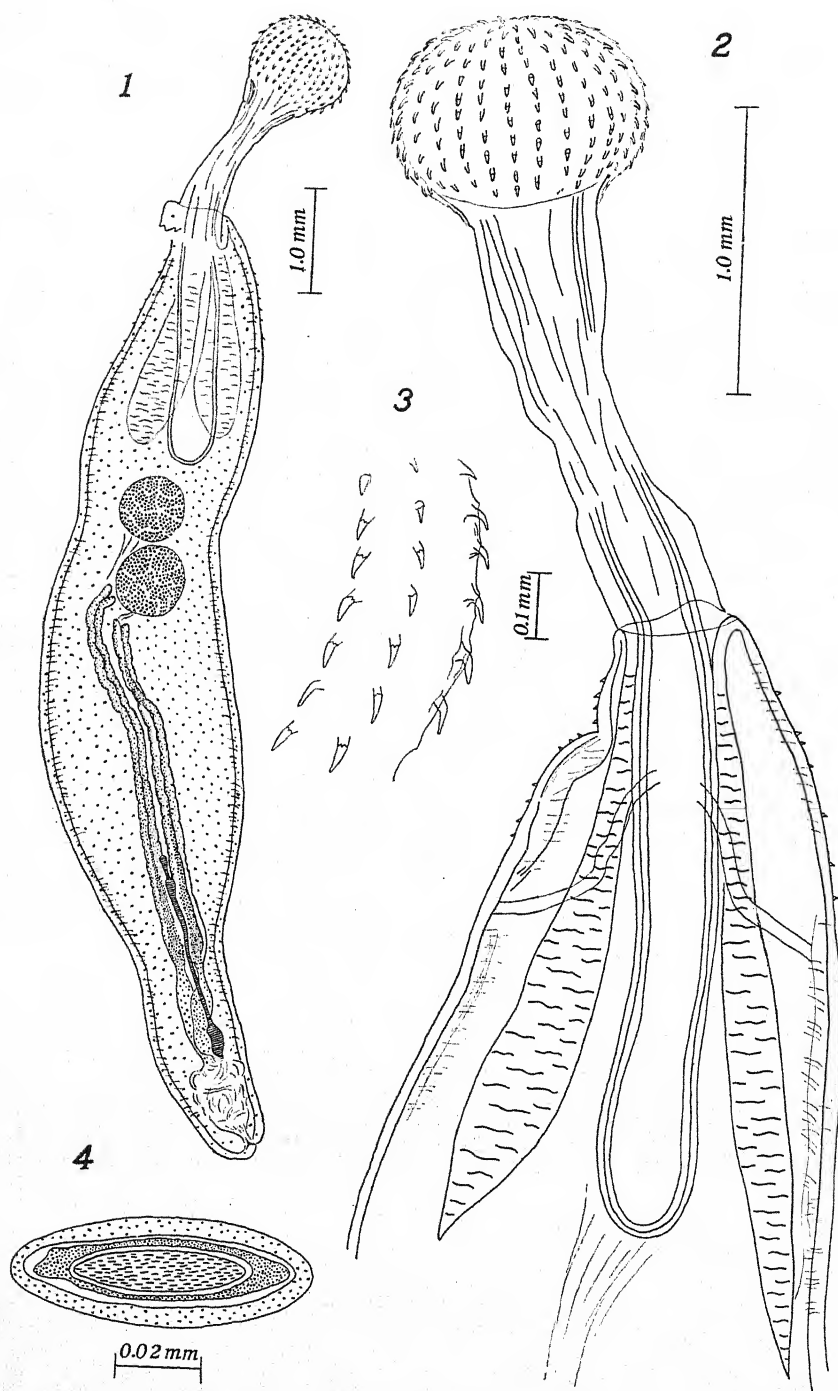


PLATE I

TRIGANODISTOMUM HYPENTELII N. SP. (TREMATODA:
LISSORCHIIDAE) FROM THE HOG SUCKER,
HYPENTELIUM NIGRICANS (LE SUEUR)¹

JACOB H. FISCHTHAL

Examination of the small intestine of a hog sucker, *Hypentelium nigricans* (LeSueur)² collected on April 3, 1941, from the Saline River near Milan, Monroe County, Michigan, revealed three specimens of a new species of trematode belonging to the genus *Triganodistomum* Simer, 1929. In the fall of 1941, two of nine hog suckers taken from the Saline River near Saline, Washtenaw County, Michigan, contained three and four adult trematodes respectively. The following description is based upon the study of living specimens, and of whole mounts and transverse sections of specimens fixed in hot Bouin's fluid, stained in Mayer's paracarmine (whole mounts) or Ehrlich's acid haematoxylin (sections), and mounted in clarite X (Nevillite no. 1).

Triganodistomum hypentelii n. sp.
(Figs. 1-4)

Description: Triganodistomum. Body long, cylindrical, arched dorsally. Suckers and ventral surface spinose; margins of body spinose to anterior edge of posterior testis, with spines on ventral surface continuing postero-mesially from this level and terminating slightly beyond posterior testis; spines posterior to acetabulum arranged quincuncially; dorsal surface spineless, except dorsal to oral sucker. Oral sucker subterminal, smaller than acetabulum; latter prominent, protruding noticeably beyond ventral surface, at anterior fourth of body. Prepharynx short, narrow, muscular; pharynx globular, very muscular; esophagus slender, muscular; intestinal bifurcation at anterior margin of acetabulum; crura slender, dorsal to vitellaria, extending nearly to posterior end of body, left cecum usually longer than right, inner walls lined with single, conspicuous layer of cells. Vitelline follicles of varying size, 28-38 per side, mostly extracecal, posterior to acetabulum from anterior margin of seminal vesicle to middle of posterior testis. Paired vitelline ducts uniting ventral to ovary; common vitelline duct short, slightly swollen.

Ovary median, post-acetabular, trilobed, appearing as three separate structures; lobes smooth, approximately equal, uniting ventrally forming a small, slightly enlarged central portion at their junction; ovary filling intracecal space, slightly overlapping anterior testis dorsally. Oviduct short. Seminal receptacle small, arising from oviduct opposite origin of Laurer's canal about midway between ovary and oötype. Laurer's canal straight, heavy-walled, leading to opening on dorsum directly above anterior lobe of ovary. Mehlis' gland extensive, well-developed, of many cells. Uterus continuing slightly forward from oötype, recurving and descending to posterior part of body; coils fairly extensive, filling body as far forward as anterior margin of seminal vesicle; uterus ascending on left side of body; metraterm short, curved, muscular, thick-walled, postero-ventral to cirrus; genital atrium small. Ovary and testes arranged in series in posterior half of body.

Received for publication, December 10, 1941.

¹ Contribution from the Department of Zoology, University of Michigan.

² The names of fishes used in this report are those given in the keys of Hubbs and Lagler (1941).

Testes two, smooth, ellipsoidal, post-ovarian, approximately equal. Anterior testis slightly overlapping ovary ventrally, separated from posterior testis by uterine fold. Vasa efferentia dorsal to testes; right vas efferens leading from right antero-lateral margin of posterior testis, left from antero-medial margin of anterior testis; vas deferens short. Seminal vesicle internal, bipartite, innermost chamber about one-half as large as outer. Prostate gland cells numerous. Cirrus about two-fifths length of cirrus sac, inverted, protrusible, bearing long, slender spines at innermost portion, spines becoming abruptly shorter, more blunt toward genital pore. Cirrus sac long, curved, anterior portion slightly overlapping acetabulum dorsally, extending well posterior to latter. Genital pore ventral, almost on left margin, at level of middle of acetabulum.

Excretory pore terminal; bladder moderately thick-walled, long, tubular, bead-like in appearance, extending dorsal to testes as far as posterior fourth of ovary; primary collecting ducts forming loops dorso-lateral to oral sucker and receiving secondary collecting ducts posteriorly.

Eggs small, numerous, operculate, shell transparent; extruded freely from genital pore after placing worms in tap water; containing ciliated miracidium when deposited; no spontaneous movement visible within egg; latter not hatching in tap water. Miracidia squeezed from eggs showing presence of anterior papilla, primitive gut just posteriorly, and germ cells filling posterior half of body; cilia very long, longer than body of miracidium, arising in collar from anterior fourth of body and extending posteriorly; remainder of body devoid of cilia. Twenty eggs freshly extruded into tap water: $0.019 (0.019-0.020) \times 0.013 (0.012-0.013)$ mm.

Measurements (in millimeters with the mean out of parenthesis and the minimum and maximum in parenthesis) of 3 adult worms mounted in toto: Body, length 2.155 (2.10-2.190), width 0.427 (0.405-0.465); oral sucker, $0.153 (0.150-0.158) \times 0.160 (0.150-0.165)$; acetabulum, $0.215 (0.210-0.225) \times 0.188 (0.180-0.195)$; prepharynx, length 0.028 (0.027-0.029); pharynx, $0.083 (0.078-0.091) \times 0.095 (0.093-0.096)$; esophagus, length 0.120 (0.107-0.133); ovary, $0.228 (0.210-0.240) \times 0.166 (0.155-0.179)$; metraterm, $0.149 (0.138-0.165) \times 0.043 (0.040-0.045)$; anterior testis, $0.233 (0.210-0.250) \times 0.148 (0.131-0.165)$; posterior testis, $0.266 (0.250-0.293) \times 0.153 (0.142-0.165)$; cirrus sac, $0.350 (0.345-0.360) \times 0.091 (0.088-0.093)$; seminal vesicle, $0.137 (0.131-0.144) \times 0.081 (0.080-0.083)$; anterior end of body to anterior margin of acetabulum, $0.375 (0.330-0.405)$; posterior margin of acetabulum to posterior margin of cirrus sac, $0.262 (0.225-0.30)$; posterior margin of acetabulum to anterior margin of ovary, $0.380 (0.345-0.420)$; posterior margin of posterior testis to posterior end of body, $0.308 (0.30-0.315)$; ends of intestinal ceca to posterior end of body, $0.105 (0.096-0.115)$.

Host: *Hypentelium nigricans* (LeSueur).

Habitat: Small intestine.

Locality: Saline River, Monroe and Washtenaw Counties, Michigan.

Type: U. S. Nat. Mus. Helm. Coll. No. 44974; *paratypes* in author's collection.

T. hypentelii differs from all known species of *Triganodistomum* in having vitellaria whose anterior limits lie some distance behind the acetabulum instead of at the posterior half of it. The extension of the cirrus sac well posterior to the acetabulum differentiates it further from *T. attenuatum*, *T. mutabile* and *T. simeri*. It differs further from *T. mutabile* and *T. simeri* in having approximately three times as many vitelline follicles. It is further differentiated from *T. attenuatum* in possessing a deeply trilobed ovary, and from *T. translucens* in having the acetabulum at the level of the anterior fourth of the body.

The genus *Triganodistomum* was established by Simer (1929) from 15 specimens of *T. translucens*, the type species, from the small intestine

of a smallmouth buffalofish, *Ictiobus bubalus* (Raf.) from the lower Tallahatchie River, Mississippi. To this was added *T. attenuatum* and *T. simeri* by Mueller and Van Cleave (1932) from the intestines of common suckers, *Catostomus commersonnii* (Lac.) from Oneida Lake, New York; more information on infection with these two species was given in Van Cleave and Mueller (1934). The generic diagnosis was emended and further information was supplied to the descriptions of *T. attenuatum* and *T. simeri* by Mueller (1934). On further restudy of *T. attenuatum*, Mueller (personal communication) found the cirrus to be spined, although previously it had not been described as bearing spines; he suspects that in favorable material of *T. simeri* spines might be found on the cirrus also. Wallace (1939, 1941) demonstrated that *Cercariaeum mutabile* Cort, 1918 developed into an adult *Triganodistomum*, the new combination being *T. mutabile* (Cort); the adult worms were recovered from intestines of experimentally and naturally infected western lake chubsuckers, *Erimyzon sucetta kennerlyi* (Girard), the latter from Waterloo Lake, Michigan.

The genus *Lissorchis* was established by Magath (1917) from specimens of *L. fairporti*, the type species, from the intestines of smallmouth buffalofish, *Ictiobus bubalus* (Raf.) and bigmouth buffalofish, *Megastomatobus cyprinella* (Valenciennes) from fish ponds at the Fairport Biological Station, Iowa. Wallace (1941) has drawn attention to similarities in the genera *Lissorchis* and *Triganodistomum*, and has placed the latter genus tentatively in the family LISSORCHIIDAE Poche, 1925, the validity of which must await further studies upon the related families, ALLOCREADIIDAE, PLAGIORCHIIDAE and MACRODEROIDIDAE.

KEY TO THE SPECIES OF THE GENUS *Triganodistomum*

1. Anterior limits of vitellaria posterior to acetabulum *T. hypentelii*
Anterior limits of vitellaria at posterior half of acetabulum 2
2. 7-12 vitelline follicles per side 3
16-32 vitelline follicles per side 4
3. Ovary deeply trilobed, appearing as three separate structures; length of body posterior to hind testis roughly equal to length of three gonads taken together *T. simeri*
Ovary moderately trilobed, not appearing as three separate structures; length of body posterior to hind testis roughly equal to one-fourth length of three gonads taken together *T. mutabile*
4. Body long (3.5 mm); length posterior to hind testis roughly equal to length of both testes taken together *T. attenuatum*
Body short (1.0 mm); length posterior to hind testis roughly equal to one-third length of both testes taken together *T. translucens*

BIBLIOGRAPHY

- HUBBS, CARL L. AND KARL F. LAGLER 1941 Guide to the fishes of the Great Lakes and tributary waters. Bull. Cranbrook Inst. Sc. 18: 1-100.
- MAGATH, THOMAS BYRD 1917 The morphology and life history of a new trematode parasite, *Lissorchis fairporti* nov. gen., et nov. spec. from the buffalo fish, *Ictiobus*. J. Parasitol. 4: 58-69.
- MUELLER, JUSTUS F. 1934 Parasites of Oneida Lake fishes. Part IV. Additional notes on parasites of Oneida Lake fishes, including descriptions of new species. Roosevelt Wild Life Ann. 3: 335-373.
- MUELLER, JUSTUS F. AND HARLEY J. VAN CLEAVE 1932 Parasites of Oneida Lake fishes. Part II. Descriptions of new species and some general taxonomic considerations, especially concerning the trematode family Heterophyidae. Roosevelt Wild Life Ann. 3: 79-137.
- SIMER, PARKE HARVEY 1929 Fish trematodes from the lower Tallahatchie River. Am. Midland Naturalist 11: 563-588.
- VAN CLEAVE, HARLEY J. AND JUSTUS F. MUELLER 1934 Parasites of Oneida Lake fishes. Part III. A biological and ecological survey of the worm parasites. Roosevelt Wild Life Ann. 3: 161-334.
- WALLACE, HAROLD E. 1939 Life history of *Triganodistomum mutabile* (Cort): Trematoda. J. Parasitol. 25 Suppl: 26-27.
- 1941 Life history and embryology of *Triganodistomum mutabile* (Cort) (Lissorchiidae, Trematoda). Tr. Am. Micr. Soc. 60: 309-326.

EXPLANATION OF PLATE, p. 393

- FIG. 1. *Triganodistomum hypentelii*, adult, ventral view.
- FIG. 2. Diagram of female genital system, ventral view.
- FIG. 3. Cirrus sac, showing bipartite seminal vesicle, prostate gland, inverted cirrus with spines, and genital pore; also muscular metraterm.
- FIG. 4. Operculate egg containing miracidium.

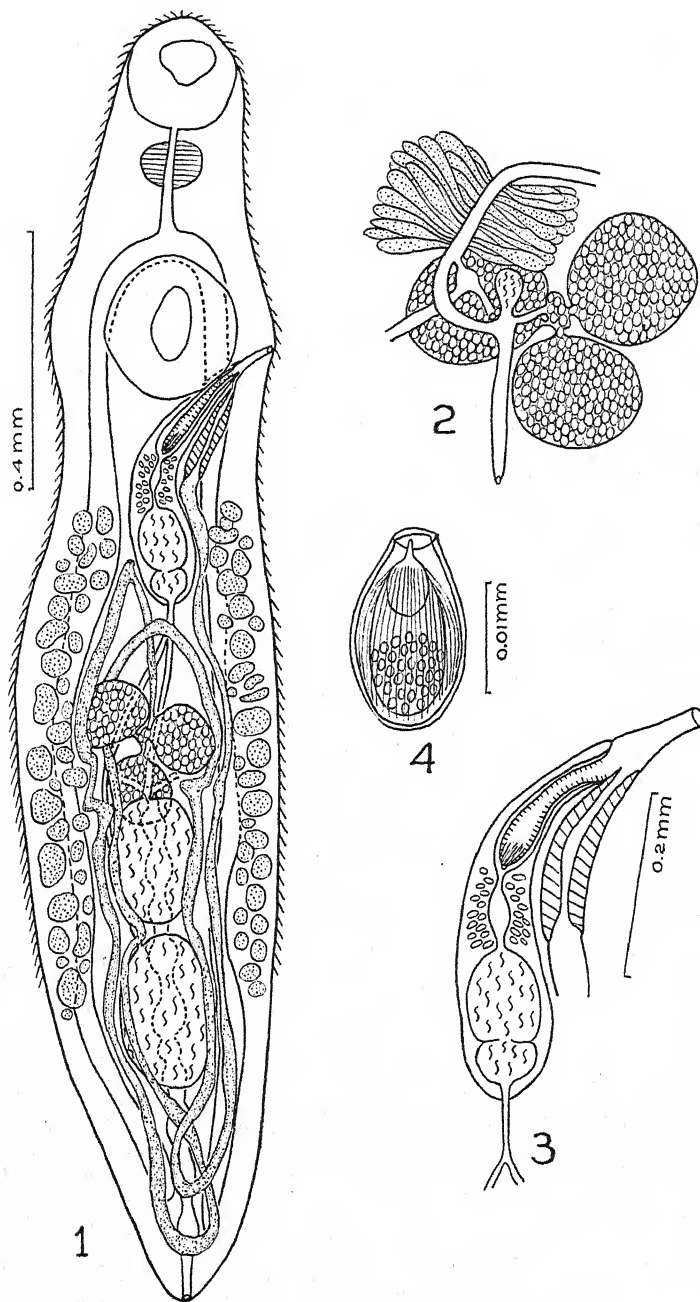


PLATE I

MECHANICAL TRANSMISSION OF RABBIT FIBROMA (SHOPE) BY CERTAIN HAEMATOPHAGOUS BUGS*

CORNELIUS B. PHILIP

Hamilton, Montana

The means by which Shope rabbit fibroma is maintained or transferred in nature is still speculative. The frequent location of the lesions about the legs and haunches in naturally infected animals suggests the possibility of some biting inhabitant of the nests or warrens acting as a vector. While there is no information of triatomid bugs infesting these locations in endemic areas in the eastern United States, the availability of laboratory colonies of certain species provided the opportunity to make simple mechanical transmission tests.

MATERIALS

It was the writer's good fortune to be located for a short period at the Johns Hopkins School of Hygiene where the facilities of the Department of Immunology were made available through the kindness of Dr. R. R. Hyde and other members of the staff to whom he is much indebted for various favors. The strains of rabbit fibroma virus used in the transmission tests, and of myxoma employed for immunity tests were those in routine use in that laboratory. The colonies of *Rhodnius prolixus* and *Triatoma infestans* were rearings from stocks obtained a few years ago during a visit of Dr. E. Dias of the Oswaldo Cruz Institute, Rio de Janeiro. The colony of *Triatoma protracta* was bred from specimens collected by Mr. Glen M. Kohls in Arizona in 1939. Domestic rabbits from a closely inbred colony were used as test animals.

EXPERIMENTAL DATA

An animal was given massive intradermal injections of virus on a clipped side and used as donor for subsequent bug feedings. A large, active fibroma developed rapidly. Nymphal and adult bugs were fed directly on the lesion on the fourth and seventh days. The first groups were labelled lots A1 and A2 and those fed on the seventh day were designated as lots B1 and B2. The A1 and B1 lots comprised insects used in alternate or interrupted feedings between the donor and a test animal at each period. The A2 and B2 lots represented bugs given initial complete feedings on the donor alone for later test after storage at

Received for publication, December 11, 1941.

* This study was conducted incidental to tenure of a Fellowship from The John Simon Guggenheim Memorial Foundation.

laboratory temperature. In three instances, additional animals were injected with saline suspensions of the viscera of insects used in prior feeding tests.

Protocols of transmission tests are given in Tables 1 and 2.

TABLE 1.—*Protocols of tests of mechanical transmission of fibroma virus by Triatomidae*

Lot	Species	Number of		Test animal	Myxoma challenge	Transmission results
		Bugs	Bites*			
A1 (4th day)	<i>T. infestans</i>	7	19	I	Survived (local lesion)	Positive; also transferred to following
	<i>R. prolixus</i>	2	3			
	Ia	Survived (no lesions)	Confirmatory transfer from I
B1 (7th day)	<i>T. infestans</i>	2	injec.	II	Survived (local lesion)	At least 1 or 2 of above 7 bugs contained virus
	<i>T. infestans</i>	3	8			
	<i>T. protracta</i>	8	29	III	Survived (local lesion)	Positive
	<i>R. prolixus</i>	2	3			
	<i>T. infestans</i>	1	injec.	IV	Survived (scattered lesions)	At least part of previous bugs contained virus
	<i>T. protracta</i>	1	"			
	<i>R. prolixus</i>	2	"			

* Certain individuals were induced to accept more than one feeding alternately on the donor and then on the test rabbit. Rabbits II and IV were injected with visceral contents of part of the bugs which bit rabbits I and III.

TABLE 2.—*Protocols of "incubation" test of fibroma transmission by stored bugs*

Lot	Species	No. biting	Interval*	Test animal	Myxoma challenge	Transmission results
A2	<i>T. infestans</i>	6	15	V	Died	Negative
B2	<i>T. protracta</i>	7	12			
B2	<i>R. prolixus</i>	1	12			
B2	<i>T. infestans</i>	7	70	VI	Died	Negative
	<i>T. protracta</i>	6	70			
B2	<i>T. infestans</i>	2†	71	VII	Died	Negative
	<i>T. protracta</i>	4†	71			

* In days after infected feeding.

† Gut contents injected.

Test rabbit I developed a small raised, inflammatory lesion at the site of insect bites beginning on the second day, attaining the size of a large garden pea on the third, and by the sixth had an erythematous area 15 mm in diameter. There being no further enlargement in the next three days, the fibroma was excised (ninth day), triturated in saline, and the extract injected intradermally into rabbit Ia. On the 45th day, no further evidence of infection having occurred, animal I was given an intradermal challenge dose of 10,000 units of myxoma virus, following which a lesion the size of a silver dollar developed at the site of injection with ultimate necrosis, but there was no further evidence of generalized infection. The animal was discarded on the 27th day after immunity test.

The transfer animal, Ia, developed a typical, though small fibroma at

the site of injection, and no reaction whatever occurred following a challenge test simultaneous with that of No. I.

Test animals II and IV developed small, subcutaneous nodules at sites of bug injection but no surface necrosis or inflammation of the skin occurred in either. On the 45th and 42nd days they received a challenge dose of myxoma virus simultaneously with rabbit I. No. II developed a local, ultimately necrotic lesion the size of a silver dollar at the site of injection and was discarded 27 days after immunity test. In No. IV, the infection became systemic with nodules on the ears, discharges from the eyes and nose, and respiratory difficulties; the animal was discarded on the 27th day, after survival and recovery were assured. It was apparent partial immunity also had been acquired by No. IV, though more feeble than in preceding animals.

Test animal No. III showed no lesions at the site of bug bites, but on immunity test on the 42nd day with myxoma, it developed a local necrotic lesion about the size of a half-dollar at the site of injection, and was discarded 27 days later. Since laboratory experience has indicated 100% fatal susceptibility of domestic rabbits to myxoma virus, it is evident that rabbits I to IV had been partially immunized by fibroma virus. (Rabbits V and a control died nine days after duplicate inoculums.)

Test animals V, VI, and VII showed no reactions following initial test, and died in 9, 10 and 10 days after immunity test, respectively. The myxoma inoculum of V duplicated the dosage given rabbits I to IV, while rabbits VI and VII also received 10,000 units of a later preparation of myxoma virus.

COMMENTS

It is well known (Shope, 1932; Shaffer, 1941; and others) that infection with fibroma virus immunizes against the invariably fatal myxoma virus in the domestic rabbit. Consequently, the latter can be used in immunity tests of fibroma-recovered rabbits.

It is presumed that mechanical transmissions by bugs given interrupted feedings on the donor (lots A1 and B1) were through the agency of contaminated mouth parts, by inference from experience in yellow fever studies with bedbugs (Philip, 1930) which are zoological relatives of triatomids and feed in a similar manner. In the yellow fever studies, 59 bugs of various stages did not transfer the virus during several interrupted, alternate feedings on an infected and a susceptible monkey, indicating that regurgitation of even minute amounts of ingested blood did not take place during feeding on the test animal since yellow fever virus has been shown to be infectious in extremely high dilutions.

Furthermore, it is believed in the present tests that the virus also was actually ingested, at least by the partially fed bugs used in the immediate transfer tests, so that quantitatively even more should have been imbibed

by the insects allowed to fully engorge on the donor rabbit without interruption, and stored for later "incubation" tests. The manner of feeding allowed only the inserted beaks of the bugs in contact with the fibroma, and the heads and appendages later were carefully snipped off previous to evisceration in the tests by injection so that the bugs tested in animals II and IV must have contained ingested virus to produce positive results. The negative results with incubated insects (Table 2) was not, therefore, due to failure to ingest virus during the original blood-meals.

The tests, however, are obviously deficient in many respects for conclusions regarding insect transmission of fibroma virus. In addition to the use of unnatural insect hosts, the numbers of bugs in the two positive biting transfers was larger than would occur under natural conditions at any one time. Also the interruptions of other activities prevented the desirable determination of the actual duration of the virus in the bugs by an adequate number of intermediate tests. It seems not unlikely that some other species of biting insects could serve to transfer the virus at least in a similar mechanical manner. Whether arthropods of any species contribute to natural maintenance or transfer of fibroma virus remains to be demonstrated. If direct feeding on active fibromatous lesions is prerequisite to parasitic transfer of the virus, it is difficult to visualize the mechanism responsible for observed natural incidence of the disease. These experiments merely suggest one possible method of mechanical transfer.

SUMMARY

Two transmissions each of rabbit fibroma to domestic rabbits were obtained by interrupted feedings, and by injection of visceral contents of 3 species of triatomid bugs which had been fed immediately preceding on an animal infected 4 and 7 days previously. All 4 test rabbits survived immunity challenge tests with myxoma virus. Feeding tests of additional simultaneously, fully fed bugs were negative after periods of 12, 15 and 70 days storage at laboratory temperatures, as was also a test by injection on the 71st day.

It is considered probable that mechanical transfer was by the contaminated mouth-parts rather than by regurgitation, and that in addition the virus was actually ingested by the bugs.

BIBLIOGRAPHY

- PHILIP, C. B. 1930 Possibility of mechanical transmission by insects in experimental yellow fever. *Ann. Trop. Med. and Parasitol.* 24: 493-501.
SHAFFER, J. G. 1941 Antigenic relationship of infectious myxoma and fibroma viruses of the rabbit. *Am. J. Hyg.* 34 B: 102-120.
SHOPE, R. E. 1932 A filtrable virus causing a tumor-like condition in rabbits and its relationship to *Virus myxomatosum*. *J. Exper. Med.* 56: 803-822.

FURTHER OBSERVATIONS ON *PLISTOPHORA KUDOI*, A MICROSPORIDIAN OF THE COCKROACH*

VICTOR SPRAGUE AND JUANITA RAMSEY

Marshall College, Huntington, West Virginia

In the mid-gut of *Blatta orientalis* Linnaeus, collected from time to time on the campus of the University of Illinois, Urbana, Illinois, in the summers of 1937-1940, the senior author occasionally noticed protozoan spores, apparently of a microsporidian. This parasite may be identical with one of undetermined nature observed by Perrin (1905), although the spores he saw, being about 1-2 μ long by 0.5 μ wide on permanent preparations, were somewhat smaller than those described below in the living condition.

In the summer of 1941 the authors had access to the laboratory facilities of the Department of Zoology of the University of Kentucky, where a further study of the organism was made and its microsporidian nature confirmed. Since recent literature, including Kudo (1924) and Jirovec (1936), indicates no similar organism, it was described as a new species in a preliminary note (1941), and the name *Plistophora kudo*i proposed in honor of Professor R. R. Kudo.

MATERIAL AND METHODS

The host insects employed for the present study were collected on the campuses of Marshall College, Huntington, West Virginia, and the University of Kentucky, Lexington, Kentucky, in May and June, 1941, respectively. Pending examination they were kept in the laboratory in battery jars and fed a diet of apples.

The mid-gut and ceca of the host were extracted in 0.75 per cent NaCl solution. The organism was then studied in the living condition and later in stained smears or sections. The fixing solutions of Carnoy, Schaudinn, and Bouin were used with equally good results. The smears and sections were either stained with Heidenhain's iron-haematoxylin or treated by Feulgen's method. Good preparations were obtained by both methods; but the Feulgen reaction was not found particularly useful, since the nuclei of the parasite are so small that it is often hazardous to draw conclusions

Received for publication, December 13, 1941.

* Contribution from the zoological laboratories of the University of Kentucky, and Marshall College. The authors acknowledge their indebtedness to Dean William D. Funkhouser, Dr. Alfred Brauer, and Dr. Marvin C. Meyer for the courtesies extended in the use of the laboratory facilities of the University of Kentucky; and to Professor R. R. Kudo, of the University of Illinois, for authoritative advice during the course of the investigation.

regarding them. No attempt is made, therefore, to give a complete account of the life cycle in the present paper.

For demonstrating the polar filament, the structure was extruded mechanically and observed with dark field illumination according to the method of Kudo (1921).

OBSERVATIONS

Host-Parasite Relation

The incidence of infection is high in the localities mentioned above. Ten adult individuals collected in Huntington and examined a few weeks later were all infected. Twenty-nine of 42 adult individuals collected in Lexington and examined over a period of three weeks harbored the parasite. Thus, the microsporidian was observed in 75 per cent of all the host insects examined. This percentage applies about equally to males and females. Possibly some of the insects acquired the infection in the laboratory by close contact with those already infected. This incidence cannot, therefore, be regarded as truly indicative of the incidence in nature. Of 17 hosts examined in Lexington the next day after collection, however, 14 were infected. Thus, the incidence under natural conditions in this locality is approximately that given above.

A heavy infection can usually be detected by examining the ceca with the aid of a dissecting microscope. If the infected ceca happen to be distended with fluid, large opaque masses of spores are often seen in the epithelial lining. The masses tend to be arranged in longitudinal rows corresponding to the longitudinal plicae which occur in the epithelium, for the infection is the heaviest in the cells along the crests of these plicae. It is suggested that these particular cells are most heavily parasitized because their situation renders them most readily accessible to the invading organism. When a cecum contains a particularly heavy infection, great numbers of the epithelial cells are sloughed off and these, with the parasites, are seen as opaque masses lying free in the lumen.

The spores of the microsporidian are hardly discernible through the intestinal wall with a dissecting microscope. The gut contents, surrounded by a peritrophic membrane, can usually be removed very readily, however, and the masses of spores appear, with reflected light, as small white objects associated with the peritrophic membrane.

The relationship of the microsporidian to the host is one of true parasitism, for obvious damage is done to the epithelial tissue. Heavily infected cells are often greatly hypertrophied. The cytoplasm may become almost completely replaced with spores, and the distended cell membrane forms a sac which extends far out beyond the neighboring cells (Fig. 39) so as to resemble a budding process. The distal ends of these hypertrophied cells, packed with countless spores, often appear to break off and pass out into the lumen (Fig. 38). This process was noted with particu-

lar frequency in the ceca, where the lumina often contain enormous numbers of these remnants of epithelial cells packed with spores. Very frequently, however, the entire host cell is sloughed off, for a nucleus can often be seen within. Thus, large areas of epithelium, particularly that on the crests of the plicae in the ceca, become severely damaged.

Regarding the hypertrophy of the epithelial cells and the sloughing of parts of their substance, the phenomenon may be due largely to a mechanical process rather than to a specific tissue reaction. The hypertrophied cells typically contain such great numbers of spores that their bulk cannot be contained within the limits of a cell of normal size. Furthermore, cells which contain many parasites, but not in sufficient quantity to distend the cell bodies mechanically, are not noticeably hypertrophied (Fig. 40). Finally, the nuclei, which seem never to be parasitized, apparently show no specific reaction. Nuclei of infected cells and those of normal cells are the same in size and appearance (Figs. 39 and 40). In this respect the microsporidian differs from the majority of known forms. In sloughed off cells, of course, the nuclei undergo degeneration.

In summarizing the effect of the microsporidian on the host, it may be safely stated that considerable damage is done to the epithelial cells lining the ceca and mid-gut. No other effects, either microscopic or macroscopic, are evident. Whether the infection is ever fatal to the host is uncertain, for no data on this question are at hand.

The Spore

The mature spores in the fresh condition are about $3.2\ \mu$ long by $1.75\ \mu$ broad, highly refractive, reniform, pyriform or ovoid, and circular in cross section. At a relatively low magnification all the spores look essentially alike, but with high magnification much variation in size is seen (Figs. 1-7). Although the majority are approximately $3.0\ \mu$ in length, a few are as long as $5.0\ \mu$. These long spores are scarcely any greater in diameter than the smaller ones, for this dimension does not seem to vary so much as the length. The typical shape is ovoid, though the anterior end is usually not plainly attenuated (Figs. 1 and 2). Upon close examination, however, many appear reniform (Figs. 5-7) and others pyriform (Figs. 3 and 4). In size and shape these spores greatly resemble those of *Thelohania reniformis* described by Kudo and Hetherington (1922). Distinct dimorphism does not seem to occur, for all intermediate conditions of size and shape seem to exist between the extremes. Moreover, much variation of spores in the same sporont is sometimes seen (Fig. 10).

Internally, the mature spores in the fresh condition appear absolutely structureless (Figs. 1-7). This is due, no doubt, to a relatively thick membrane, which also accounts for the great refractivity. Young spores, on the other hand, are much less refractive and contain a conspicuous

translucent area near one end resembling a vacuole (Figs. 8 and 9). This area is colorless in stained spores (Fig. 30).

The presence of a polar filament was easily demonstrated many times by Kudo's pressure method, as mentioned above. The filament is very delicate, uniform in diameter along the entire length, and relatively short (Fig. 29). Its length is approximately $25\ \mu$ in an apparently fully extruded condition. Rarely a filament as long as $50\ \mu$ was seen. Four to six undulations are often apparent in the filament, corresponding, no doubt, to the coiled condition existing before extrusion.

Developmental Stages

The stages preceding the schizont were not recognized. Presumably, the sporoplasm emerges from the spore membrane after ingestion by the host, makes its way to the specific site of infection, and eventually gives rise to the schizont stage. The authors believe the latter to be represented by certain elongated bodies (Figs. 11 and 37) which are very common in the cytoplasm of the epithelial cells. These objects are cylindrical, sausage-shaped, clavate, or fusiform and contain as many as ten small massive nuclei. The latter are usually arranged in a linear series and spaced at more or less equal intervals; sometimes, however, they are somewhat irregularly arranged. The infected cells may contain one to many schizonts. In the latter case, all the schizonts typically lie parallel in a compact group. They frequently lie also parallel with the long axis of the cell, due, perhaps, to mechanical causes.

Schizogony apparently results in the formation of small spherical bodies (Fig. 12), each with a single massive nucleus and with cytoplasm having somewhat less affinity for haematoxylin than the parent schizonts. Whether schizogony ever gives rise to multinucleate products was not determined. It is also not certain whether the schizogonic multiplication may be repeated, but it seems probable that it may. This inference is supported by the fact that cells containing many schizonts occur with great frequency. Repeated schizogony is a more plausible explanation of this frequency than multiple initial invasion.

The processes which initiate the sporogonic cycle are likewise not clear. Presumably, the young sporonts arise from the products of schizogony, but no distinguishing characteristics which might aid in identifying them were observed. Occasionally, small binucleate bodies as well as uninucleate ones were seen (Fig. 13). These may be young sporonts; they might also represent stages in schizogony or some other process. At any rate, whatever the initial stages may be, later stages are easily recognized. The young schizont develops by repeated nuclear division and simultaneous increase in the size of the protoplasmic body (Figs. 14-18). Frequently, a strand connects the two products of nuclear division

for a time (Figs. 17, 18, and 37). The cytoplasm of the developing sporonts has greater affinity for haematoxylin than that of the host cells or of other trophic stages of the parasite. Furthermore, the sporont, particularly if it is a larger one, characteristically lies in an apparent vacuole (Fig. 37) and is thus sharply set off from the host cytoplasm. This is not true of the other stages; thus they are studied with more difficulty because their boundaries are indistinct.

Eventually, the nuclear division ceases and the cytoplasm, which was formerly uniformly dark and homogeneous in appearance, begins to be differentiated into dark and light portions. The darker cytoplasm condenses around the nuclei, this being the beginning of sporoblast formation (Fig. 19). Later, the sporoblasts acquire their definitive ovoid shape and each, as seen in Heidenhain preparations, appears to have a single massive nucleus in the center of a colorless circular area near one end. These young sporoblasts are observed more readily in smears (Fig. 20) than in sections (Figs. 21 and 40), for in the latter they are usually closely packed together and are spherical in appearance. The residual protoplasm of the sporont, when it is apparent, is colorless and its limiting membrane distinct.

When observing the stages in the development of the sporoblast into a mature spore, one encounters great difficulties due to the extreme smallness of the objects. Apparently, as mentioned above, the young sporoblast is uninucleate at one stage. Older sporoblasts, as viewed in Heidenhain preparations, frequently appear to have more than one nucleus (Figs. 22-26). It was not possible to follow the stages in development, but it is probable that this process is accompanied by a complicated series of nuclear changes. Presumably, the various nuclear elements have different functions associated with the development of the various parts of the spore. In the mature spore a single dark-staining granule (nucleus?) is very frequently seen lying in the colorless area at the posterior end (Figs. 27 and 28). Except for the one area the mature spore typically stains very darkly (Fig. 30). In some cases, however, there is a small colorless area at the opposite end (Fig. 27).

The type of sporogony is that characteristic of the genus *Plistophora*, in which, according to Kudo (1939), the "sporont develops into variable number of (often more than 16) sporoblasts, each of which becomes a spore." Two to many spores develop in each sporont in the species under consideration (Figs. 31-36). The former number is rare, for only one of this type was observed. Those containing six, eight, ten, or twelve spores are common, but the majority contain sixteen or more. The size of the spores is not noticeably correlated with the number present within a sporont.

The mature sporonts are typically spherical and vary in diameter from

5 μ to 15 μ , the average being about 8 μ in the living condition. The diameter corresponds roughly to the number of sporoblasts or spores contained, but this correspondence is far from exact. When the number of spores is large, the interior of the sporont is usually completely filled and the spores lie closely applied to the limiting membrane (Figs. 35 and 36). When, on the other hand, the number is small, there is usually a rather wide zone of very hyaline and homogeneous protoplasm between the enclosed cluster of refractive spores and the distinct sporont membrane. Thus, because of the excess protoplasm in some sporonts, the diameter does not correspond closely to the number of spores within.

SUMMARY

Seventy-five per cent of 52 specimens of *Blatta orientalis*, collected at Huntington, West Virginia, and Lexington, Kentucky, contained *Plistophora kudoï* in the epithelial cells of the ceca and mid-gut.

The mature living spores are typically ovoid, often reniform or pyriform, very refractive, and show no internal structure. They measure about 3.2 μ long by 1.75 μ in diameter, but the size is highly variable. Distinct dimorphism does not seem to exist. A conspicuous colorless area is seen in the posterior end of stained spores. The polar filament is about 25 μ long.

The schizonts are elongate, being cylindrical, sausage-shaped, clavate, or fusiform. Schizogony results in several spherical uninucleate products, some of which give rise to sporonts.

Sporonts are subspherical when immature. At maturity they are spherical and contain a variable number of spores. Usually sixteen or more spores develop, this number being characteristic of the genus *Plistophora*. The membrane is often distinct and persistent until the enclosed spores reach maturity.

The only other known species of *Plistophora* in *Blatta orientalis* is *Plistophora* sp. Mercier, 1908, which has a different site of infection and much larger spores. These and other definite morphological differences justify the conclusion that the two species are distinct.

BIBLIOGRAPHY

- JÍROVEC, O. 1936 Studien über Microsporidien. Mem. Soc. Zool. Tchécoslovaque Prague 4: 1-75.
- KUDO, R. R. 1921 Notes on *Nosema apis* Zander. J. Parasitol. 7: 85-90.
- 1924 A biologic and taxonomic study of the Microsporidia. Illinois Biol. Monogr. 9 (2-3): 1-268.
- 1939 Protozoology. Charles C. Thomas, Springfield, Illinois. 689 pp.
- KUDO, R. R. AND HETHERINGTON, D. C. 1922 Notes on a microsporidian parasite of a nematode. J. Parasitol. 8: 129-132.
- MERCIER, L. 1908 Néoplasie du tissu adipeux chez des blattes (*Periplaneta orientalis* L.) parasitées par une microsporidie Arch. Protistenk. 11: 372-381.

- PERRIN, W. S. 1905 Preliminary communications on the life-history of *Pleistophora periplanetae* Lutz and Splendore. Proc. Cambridge Phil. Soc. 13: 204-208.
- SPRAGUE, VICTOR AND RAMSEY, JUANITA. 1941 A preliminary note on *Pleistophora kudo* n. sp., a microsporidian parasite of the cockroach. Anat. Rec. 81 (4 Suppl): 132-133.

EXPLANATION OF PLATE, P. 406

All figures were drawn with the aid of a camera lucida. Figs. 38 and 39 are magnified approximately 700 times and others 1500 times. Figs. 1-9, 29, and 31-36 are from living material; Figs. 10, 19-20, and 22 are from Schaudinn-Heidenhain smears; Figs. 11-18, 21, 23-28, 30, 37, and 40 are from Bouin-Heidenhain sections; Figs. 38 and 39, Schaudinn-Heidenhain sections.

- FIGS. 1-7. Mature spores of various size and shape.
- FIGS. 8-9. Young spores with "vacuole" in posterior end.
- FIG. 10. Sporont in which the spores vary greatly in size and shape.
- FIG. 11. A schizont.
- FIG. 12. End products of schizogony.
- FIG. 13. Binucleate stages of uncertain significance.
- FIGS. 14-18. Stages in development of the sporont.
- FIGS. 19-26. Stages in development of the sporoblast.
- FIG. 27. A mature spore.
- FIG. 28. A mature spore in end view.
- FIG. 29. A spore with extruded polar filament.
- FIG. 30. A mature sporont containing twelve spores.
- FIGS. 31-36. Mature sporonts of various sizes, containing different numbers of spores.
- FIG. 37. A host epithelial cell containing various stages of the parasite.
- FIG. 38. A sloughed-off portion of an epithelial cell packed with spores.
- FIG. 39. A number of epithelial cells from one of which a portion containing spores is being sloughed off.
- FIG. 40. A cell packed with sporoblasts compared with normal ones to show that there is no change in the nucleus and little in the cytoplasm except in extreme cases.

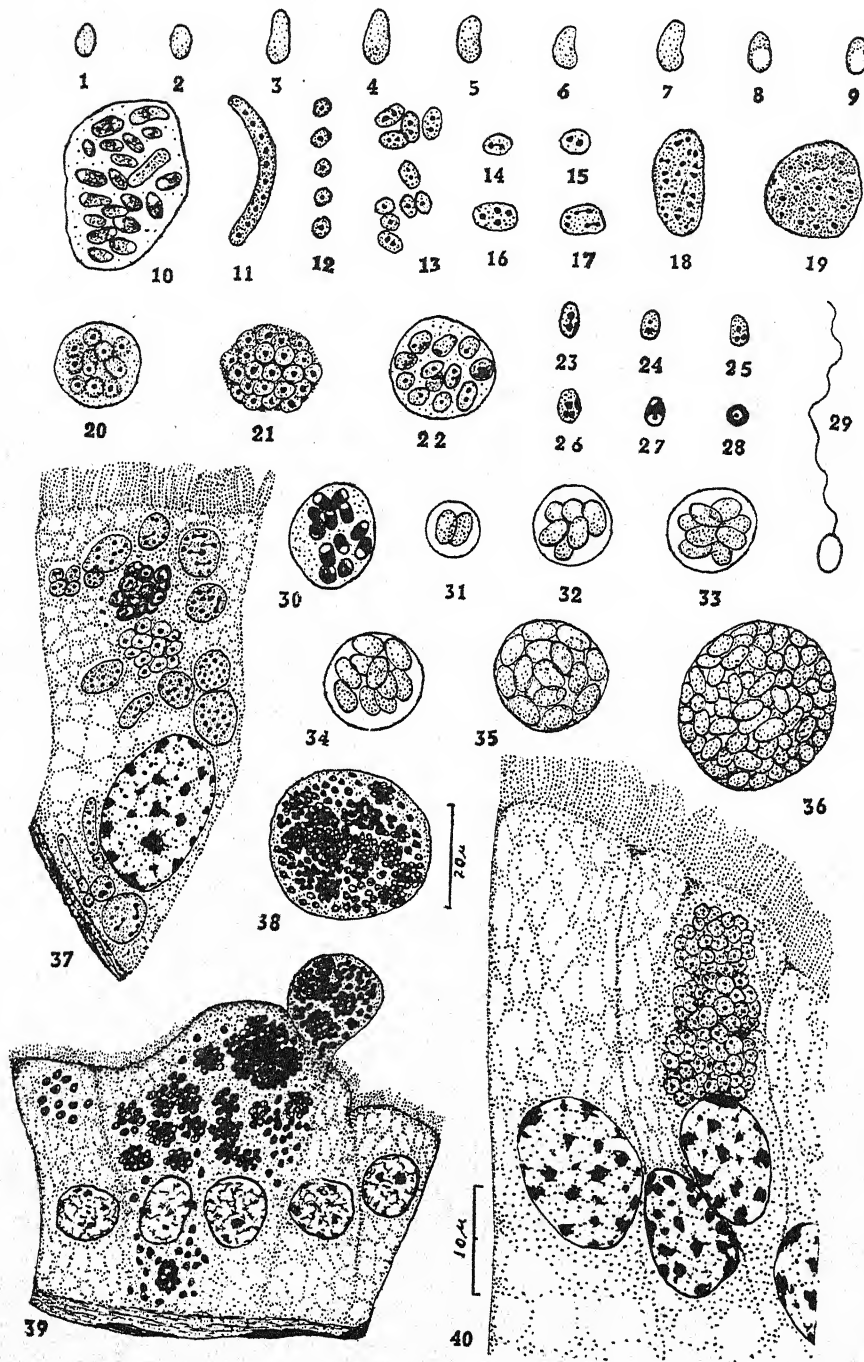


PLATE I

STUDIES ON THE LIFE HISTORY OF *SIPHODERA*
VINALEDWARDSII (LINTON) (TREMATODA:
CRYPTOGONIMIDAE)

R. M. CABLE AND A. V. HUNNINEN*

Purdue University, Oklahoma City University and the Marine Biological Laboratory

During the summer of 1939, an undescribed pleurolophocercous cercaria of an unusual type was found in the small marine snail, *Bittium alternatum*, collected from Waquoit Bay, Cape Cod, Massachusetts. This cercaria has proved to be the larva of *Siphodera vinaledwardsii* (Linton, 1901), a common parasite of the toadfish, *Opsanus tau*, in the Woods Hole region. The cercariae encyst in flounders, killifish, and possibly other fishes which may be eaten by the toadfish. The present study throws considerable light on the affinities of *Siphodera* and related genera to other trematodes, a matter concerning which there has been some uncertainty.

S. vinaledwardsii was first described as *Monostomum vinaledwardsii* by Linton (1901) who observed the ventral sucker but interpreted it as a "genital acetabulum" and therefore a part of the copulatory apparatus. The species was subsequently recorded by Linton from hosts other than *Opsanus tau*: *Orthopristis chrysopterus* at Beaufort, North Carolina (1905); *Neomaenid synagris* and *Ocyurus chrysurus* at Bermuda (1907); and again from *O. chrysurus* at Dry Tortugas (1910). In the last paper, Linton proposed the genus *Siphodera* for the species, emended its description and erected the family SIPHODERIDAE to include besides *Siphodera*, the new genera *Genelopa*, *Prodistomum*, *Stegopa* and *Metadena*. Manter (1934) expressed the opinion that these genera are not closely related and proposed the subfamily SIPHODERINAE, family HETEROPHYIDAE, to include *Siphodera* and a new genus, *Siphoderina*. Manter (1926) had previously redescribed *S. vinaledwardsii* from preserved material collected from toadfish at Woods Hole. In a revision of the superfamily OPISTHORCHIOIDEA, Price (1940) has assigned the subfamily SIPHODERINAE to the family CRYPTOONIMIDAE. From serological studies, Wilhelmi (1940) has obtained evidence supporting the view that *Siphodera* is closely related to the heterophyids.

Some of the unusual characteristics of the cercaria of *S. vinaledwardsii* have been described recently for other opisthorchioid species. These include *Cercaria coronanda* Rothschild (1938) and the cercariae of *Centrocestus armatus* as described by Yamaguti (1938), *Caecinicola parvulus*

Received for publication, January 16, 1942.

* This work was assisted by a grant-in-aid to the junior author from the Society of the Sigma Xi.

by Lundahl (1939, 1941) and *Exorchis oviformis* by Komija and Tajimi (1940).

MATERIALS AND METHODS

Large numbers of snails were collected from seaweed near the shore of Waquoit Bay, near Menauhant, and isolated in finger bowls to obtain cercariae. Flounders were used almost exclusively to observe cercarial penetration and obtain metacercariae for study and experimental feeding; the larvae penetrated these fishes more readily than other species tried. Furthermore, the flounders would lie quietly with the fins well exposed so that penetration could be observed continuously with the microscope. In some cases, fishes were exposed only one day to cercariae so that all metacercariae were of the same age; others, particularly those to be fed to toadfish, were exposed to cercariae for several days to obtain massive infections. Flounders to be infected experimentally were first examined carefully with the dissecting microscope and any containing cysts or unusual pigment spots in the fins or skin were discarded. While a few cysts might have been present in the deeper tissues, it was established by dissecting a number of fish that if infected at all, there almost always were a few cysts in the fins. Considerable difficulty was experienced in keeping experimental flounders alive until the metacercariae were infective for the definitive host. Since it was impossible to obtain parasite-free toadfish, those used in experiments were isolated for at least one month so that experimental infections could be differentiated from natural infections by differences in the degree of maturity of the worms recovered.

Throughout the study, living material was used as much as possible although stained whole mounts of larval and adult stages and sectioned adults were used as supplementary material. All figures except those indicated as free-hand drawings are to scale and were made with the aid of a camera lucida. Measurements are in millimeters.

OBSERVATIONS

Experimental Proof of the Life Cycle

Before an attempt was made to determine the life cycle experimentally, metacercariae, believed to be those of *S. vinaledwardsii*, were found in the cardiac muscle of *Paralichthys dentatus*. These later proved to be identical with larvae found encysted in the fins and beneath the scales of the fish. The flame cell formula was the same as that of the cercaria but the excretory bladder was far more extensive, being Y-shaped, with arms reaching the oral sucker as in the adult *S. vinaledwardsii*. In experimentally infected fish, it was observed that the relatively small bladder of the cercaria developed in a few days to the extent observed in metacercariae from naturally infected flounders. Furthermore, the terminal genitalia-ventral sucker complex characteristic of *Siphodera* became well developed in metacercariae from experimentally infected fish.

In a preliminary experiment, a small flounder (*P. dentatus*) containing many 9-day metacercariae was fed to a toadfish which was killed and examined four days later but found to contain only a few mature worms from previous infections. Thus it seemed that 9-day metacercariae were not sufficiently developed to establish themselves in the definitive host. Another small flounder that had been exposed to cercariae continuously for seven days, was kept alive until the oldest metacercariae were 13 days old. This fish was cut into three pieces and fed to three toadfish, one receiving one-half of the fish, the others one-fourth each. The next day, one of the toadfish fed one-fourth of the flounder was killed and well over 100 very young *Siphodera* were recovered. Size variation of the worms corresponded with the differences in the ages of the metacercariae fed. In view of the previous experiment indicating that 9-day metacercariae were not infective, it seems very likely that well over one-half of the worms recovered would not have been present had the fishes been kept four days before examination.

Three days after feeding, the second toadfish, receiving one-fourth of the flounder, was killed and found to contain 30 small, immature *Siphodera* which were more uniform in size than in the first fish examined. Presumably, worms that had been encysted less than 10 days, failed to establish themselves. The third toadfish was killed 11 days after receiving one-half of the infected flounder. About 100 (95 were separated from the debris and fixed) young and 11 adult *Siphodera* were recovered. From none of three control toadfish were as many or nearly as young *Siphodera* recovered as from any of the experimental animals.

The morphological and experimental evidence, indicating that the life history of *S. vinaledwardsii* has been demonstrated conclusively, is supported by the fact that during an extensive survey of adult trematodes in the vicinity of infected snails we have not found any species whose larval stages might be confused with those of *Siphodera*.

DESCRIPTION OF STAGES IN THE LIFE HISTORY OF *S. vinaledwardsii*

The Adult

(Figs. 1-7)

The adult worms occur in large numbers throughout the intestine of the toadfish, *Opsanus tau*. Extended observations of both living and fixed specimens make it desirable to supplement the descriptions given by Linton (1901, 1910) and Manter (1926), particularly in respect to the excretory and reproductive systems.

The writers' specimens from naturally infected toadfish are somewhat smaller than those described by Linton and Manter but these differences are not significant since our specimens were not flattened. Six repre-

sentative mature adults, fixed in Bouin's fluid after shaking in saline, and stained with Mayer's paracarmine, have the following measurements: body length 0.93–1.19 (average 1.09), maximum width 0.48–0.65 (0.56); width of oral sucker 0.14–0.17 (0.156), pharynx 0.07–0.09 (0.08); diameter ventral sucker 0.07–0.09 (0.083), ventrogenital pit 0.08–0.11 (0.093), prostatic bulb 0.08–0.10 (0.085).

The body is thick and somewhat flattened, entirely covered with fine spines, and widest behind the middle. The anterior end is broadly rounded in fixed specimens but appears more pointed in living ones. The shape of the posterior end is characteristic (Fig. 1), always being decidedly pointed and occupied largely by the excretory vesicle. The median ventral surface of the hindbody bears a prominent longitudinal furrow, the depth of which depends on the degree to which the excretory vesicle is distended. There are numerous unicellular glands with ducts opening at the surface of the body. These are especially abundant in the forebody but a few may be scattered as far back as the opening of Laurer's canal. The granular contents of the glands stain intensely with eosin.

The small ventral sucker lies in a pit-like cavity, here called the ventrogenital pit, which opens to the ventral surface. This opening is variable in size and shape and the ventral sucker may be protruded through it. The genital pore is situated on the anterior wall of this cavity. The strong muscles radiating from the ventrogenital pit were described by Linton (1910) and Manter (1926) but these authors overlooked the conspicuous zone of gland cells surrounding the pit and apparently emptying into it. Also, in both living and stained specimens, the lining of the pit is distinctly cuticular in nature (Figs. 2–3), and is provided with conspicuous spines. These spines are continuous in distribution with those of the body surface and their points are directed towards the opening of the pit where they blend into the body spines (Fig. 3).

The excretory system (Fig. 7) is simple and can be observed readily in living, immature specimens under light coverglass pressure. The excretory vesicle is ventral in position, expanded, and Y-shaped. The stem is intercecal and widest posteriorly, narrowing somewhat anteriorly; the wall is irregularly crenulate. At the level of the ovary, the vesicle divides to form the voluminous arms which extend forward, intracecally at first, pass beneath the ceca at the esophageal level, and terminate as expanded sacs near the oral sucker. The convoluted main excretory tubules enter the arms of the bladder at a level just behind the ventral sucker, extend laterally a short distance, and divide to form anterior and posterior collecting tubules. Each collecting tubule receives two secondary tubules, each of which is joined by the capillaries of two flame cells, one dorsal and one ventral in position. The flame cell formula is ac-

cordingly $2[(2+2) + (2+2)] = 16$ flame cells. The excretory pore is posterior and slightly ventral and connected with the vesicle by a slender canal.

Although there usually are four testes on each side, as many as six have been observed. They may be arranged one behind the other in the extracecal, dorsolateral regions of the hindbody, or one testis on either or both sides may lie median to the others and above the intestine. Beginning as an expanded, infundibuliform tube near each group of testes, a prominent vas efferens extends anteromediad, passing below the vitelline duct. The two vasa efferentia join a very short vas deferens which enters the seminal vesicle. The vesicle proper is divided into two parts of unequal length (Fig. 5), separated by a constriction. The terminal division is dorsal in position, elongate oval in shape, and provided with a thick wall, the outer layer of which is composed of cells which in optical section appear rectangular with slightly rounded outer edges. By focusing on the surface of the vesicle in living worms, these cells are seen to form a succession of transverse rings. Posteriorly, the heavy wall is constricted and continuous with the thin-walled posterior division of the seminal vesicle. This division is much longer than the anterior portion and sigmoid in shape; it extends ventrally in unflattened specimens (Fig. 1) but in flattened ones is usually deflected to the left and behind the terminal portion as shown in Fig. 5. The terminal anterior portion of the vesicle joins a prominent, spherical body, the prostatic bulb, which is situated above and slightly overlapping the anterior end of the ventral sucker. This bulb may be as large as the ventral sucker and is filled with pyriform glandular masses whose cytoplasm contains eosinophilic granules. These masses contain no nuclei and appear to be the inner ends of the large prostatic cells situated outside the bulb which is pierced by their ducts. A similar condition has been described for *Cryptogonimus*, *Caecinicola*, and *Allacanthochoasmus* by Mueller (1934). On account of its structure and position in respect to the genitalia, the bulb is interpreted as an enlarged pars prostatica. From its anteroventral margin, a short ejaculatory duct extends abruptly ventrad to join the vagina. From this junction, a short genital atrium continues ventrad to the genital pore. There is no evidence of a gonotyl.

The ovary is situated midventrally, just behind the seminal vesicle. It consists of many lobes which obscure details of the female complex. The oviduct begins at the median dorsal surface of the ovary as an expanded, funnel-like tube provided with radiating muscles. This portion of the oviduct probably corresponds to the "swallowing apparatus" described by many authors, and functions in propelling the oocytes through the oviduct. The oviduct extends dorsally and anteriorly, passing below the vitelline receptacle, and is joined by the seminal receptacle and Laurer's canal. The receptacle is large, oval or pyriform in shape,

and situated to the left of the seminal vesicle. The junction of Laurer's canal with the seminal vesicle duct and oviduct forms a cross. From this point, Laurer's canal curves dorsally and then posteriorly above the vitelline receptacle, opening mid-dorsally above the ovary. From the cross, the oviduct turns sharply ventrad and is joined immediately by the duct of the vitelline reservoir. Just beyond this point, the oviduct turns posteriorly and joins the ootype in which the egg shell forms. An inconspicuous Mehlis gland is present. The beginning coils of the uterus extend posteriorly as a series of convolutions, and, in about one-half of the specimens, are confined to the right half of the body behind the ovarian level. Near the posterior end of the body, the uterus crosses to the left half of the body, passing beneath the excretory vesicle, and extends forward as a sinuous tube to the level of the seminal receptacle. It then recrosses the body to the right side and, in mature worms, coils several times in front of the right testes before extending anteriorly to the vagina. The course of the uterus as just described was observed in about one-half of the specimens studied, the remainder having either a mirror image of this pattern or an irregular one. Of 35 mature worms selected at random, 18 had the pattern described, 16 the mirror image, and one an irregular pattern. The eggs (Fig. 6) measure 0.02 to 0.022 mm by 0.011 mm in living worms and are colorless when first formed. As they pass through the uterus, the shells gradually become yellow and then brown, imparting a striking chocolate color to the pretesticular uterine coils in living worms.

The vitellaria are confined to the lateral regions and just beneath the dorsal surface of the body between the levels of the genital pore and the anterior testes. Medially, the transverse band of dorsal follicles does not reach as far anteriorly as the lateral fields, but its roughly convex posterior margin usually extends backward farther than the lateral follicles. A transverse vitelline duct extends from each lateral field to join the heart-shaped vitelline reservoir.

The Redia

(Fig. 8)

The redia is similar to that described for other pleurolophocercous forms, being elongate, without locomotor processes, and provided with a short intestine and birth pore near the pharynx. The excretory system of one side is shown in Fig. 8. The largest rediae observed were about 1 mm long and contained no mature cercariae. Hence it seems that the cercarial embryos leave the redia to complete their development in the snail's tissues as described for certain other pleurolophocercous forms.

The Cercaria

(Figs. 9-10)

Specific diagnosis: Marine pleurolophocercous cercaria. Body bell-shaped when strongly contracted; spinose as far back as eye-spots; general pigmentation lacking;

cuticle with fine "hairs." Measurements of living specimens: body extended over 0.23 long, contracted 0.10, moderately extended 0.17–0.19 (av. 0.18). Eye-spots block-like, 0.009 wide. Tail inserted ventrally, 0.24–0.40 long, averaging 0.29 when moderately extended; coiled when at rest; fin-fold continuous from near base of tail ventrally to posterior end of body dorsally, becoming very narrow for part of its extent on both dorsal and ventral sides of the tail. Oral sucker averages 0.03 by 0.025; pharynx 0.008 in diameter, situated just behind eye-spot level; remainder of digestive system undeveloped; ventral sucker 0.006 in diameter. 7 pairs of cephalic glands with ducts passing over oral sucker in two bundles; openings 3, 4, 4, 3 in crypt above mouth. Excretory vesicle U-shaped with irregular walls of thick, granular cells. Main excretory tubules short; anterior and posterior collecting tubules each receiving the capillaries of 2 pairs of flame cells; excretory formula $2[(2+2) + (2+2)]$. Genital primordium an undifferentiated mass of cells immediately anterior to excretory vesicle. Develop in simple rediae.

Host: *Bittium alternatum* (Say).

Locality: Waquoit Bay, Cape Cod, Massachusetts, U. S. A.

The cercaria of *S. vinalewardsi* is unusual in a number of respects. Most noticeable are the insertion of the tail, the resting position of the larvae, and the presence of two instead of four bundles of cephalic gland ducts at the level of the oral sucker. In all described species of pleurolophocercous cercariae, the tail is set in a socket at the posterior end of the body, while in the present species, the tail is inserted in a slit-like opening on the ventral surface a considerable distance from the posterior end of the body. The posteroventral surface of the body is concave. The resting position (Fig. 10) is unique. In most if not all described pleurolophocercous species, the tail is sigmoid in lateral aspect when at rest and at the end of a swimming period, the larvae sink body downward through the water. In the present species, the tail curves dorsally, forming a spiral (Fig. 10) when at rest. If not disturbed, most of the larvae come to rest away from the light, either lying on the bottom or attached by the oral sucker to the bottom or side of the dish. A few rest on the side toward the light. There probably is a reversal of phototactic response with increasing age, since cercariae dissected from the snail are strongly photopositive. Swimming movements are similar to those of other pleurolophocercous larvae. If shadowed when at rest, the cercariae respond by swimming either toward or away from the light in a zig-zag path. If placed in a small container such as a Syracuse dish, they may swim back and forth across the dish one or more times before resting.

Various species of flounders, the killifish, *Fundulus heteroclitus*, and the stickleback, *Apeltes quadracus*, were tried as intermediate hosts by placing them in dishes with large numbers of cercariae. Flounders, especially *Paralichthys dentatus*, became heavily infected, the killifish obtained a few cysts, and the sticklebacks were free of metacercariae when examined. Small summer flounders were used for most of the experimental infections since the fins are transparent and the process of penetration could be observed continuously. If while swimming, the cercaria comes in contact with the fish and the oral sucker touches the epidermis

of the fin, the tail is lost instantly and floats away without further movements. After decaudation, there is a distinct cleft at the posterior end of the body. The secretion of the cephalic glands is immediately poured out around the oral sucker and the larva remains almost motionless for three to five minutes. It then begins to penetrate the epidermis slowly, with the posterior end motionless, the oral sucker and spines of the forebody apparently doing most of the work after the host's tissues are softened by the secretion of the cephalic glands. The larvae require 45 to 50 minutes to complete penetration, after which they migrate through the tissues, usually for only a short distance, and encyst within three hours. A small crater, with roughened edges persists for several hours at the site of penetration into the epidermis of the fish. In the fins, the larvae usually encyst near the fin rays although they may localize in the thin membrane between the rays. Many larvae encyst at the base of the fins and near the ventral surface of the body. They occur also in the deep tissues, including the myocardium.

Penetration is not influenced apparently by any chemotactic effect exerted beyond the epidermis of the fish since cercariae may swim against the fish or get beneath a fin and swim about for some time without penetrating. A definite contact between the epidermis of the fish and the oral sucker of the cercaria provides the stimulus necessary for penetration.

Metacercariae

(Fig. 11)

The young metacercaria is enclosed in a thin, oval cyst membrane of parasite origin. Dense, secondary cyst layers of host origin were not observed, nor was there any marked pigmentation around metacercariae two weeks of age. However, old cysts in the fins of naturally infected flounders were surrounded by a densely pigmented area. After encystment, the larva grows very rapidly for several days. The metacercaria at an age of less than one hour measures 0.115 by 0.09 mm; 24-hour metacercariae 0.145 by 0.12 mm; 2-day cysts 0.21 by 0.15 mm; 7-day cysts 0.27 by 0.18 mm; and six 9-day metacercariae averaged 0.28 by 0.19 mm. Shortly after encystment the body becomes opaque and filled with large vacuole-like cells (Fig. 11a). At the end of 48 hours, these cells are more numerous but more uniform in size (Fig. 11b). In 7-day metacercariae (Fig. 11c), such cells are still present but in smaller numbers and many features characteristic of the adult can be discerned. The intestinal ceca are developed, the arms of the excretory vesicle extend to the pharyngeal level and the characteristic ventrogenital pit complex is clearly seen. Although apparently in an advanced stage of development at 9 days of age, metacercariae must be at least 10 days old to be infective, as indicated by feeding experiments.

DISCUSSION

Although Witenberg (1929) clearly defined the gonotyl and ventro-genital cavity or pit, these terms have been misapplied to a considerable extent. The ventrogenital pit of *Siphodera* and related genera has been called also a gonotyl, genital sinus, genital atrium, genital sucker, pseudo-sucker and genital pit. Sections of *S. vinaledwardsii* clearly demonstrate not only that its cuticular lining is similar to and continuous with the cuticle of the body surface (Figs. 2-3) but also that it bears spines which differ only in size from those of the body. Cross sections of worms show clearly a continuous distribution and overlapping arrangement of the spines extending from the ventral sucker to the exterior body surface. The ventrogenital pit of *Siphodera* therefore is a secondary invagination of the body wall and not a genital atrium or gonotyl.

Certain genera of CRYPTOAGONIMIDAE, as described by Mueller (1934), provide an interesting and significant series of modifications of the terminal genitalia-ventral sucker complex. Of these, *Caecinicola* seems to be the most primitive since it has neither a ventrogenital pit nor a gonotyl; in this genus, the relationship of the genital pore and ventral sucker to the body surface is essentially the same as in the ACANTHOSTOMIDAE, OPISTHORCHIIDAE and many other families of digenetic trematodes. The next in the series is the genus *Allacanthochoasmus* in which a papilla-like gonotyl develops in front of the genital pore. In *Cryptogonimus*, the gonotyl is much more prominent, its base is pierced by the genital atrium, and the beginning of a ventrogenital pit is evident as an invagination of the body wall. The pit is well formed in *Siphoderina* and *Siphodera*. Since *Siphoderina* has a distinct gonotyl, its absence in *Siphodera* may be a secondary condition although the histological structure of the pit does not support that view.

In the HETEROPHYIDAE and CRYPTOAGONIMIDAE, then, the tendency of the body to become invaginated in the region of the genital pore is expressed in varying degrees. The nature and contents of the resulting pit depend on the proximity and relationship of the genital pore to the ventral sucker. Thus in *Heterophyes*, the invagination forms a genital pit independent of the ventral sucker and enclosing only the sucker-like gonotyl, while in *Cryptocotyle* and many other genera, the invagination forms a ventrogenital pit, which, as its name implies, encloses both the gonotyl and ventral sucker. The genital pore and its associated gonotyl may be either anterior or posterior to the ventral sucker which becomes greatly reduced in certain genera, being merely a muscular thickening in the wall of the ventrogenital pit.

There also is an interesting series in the relationship of the genital pore to the gonotyl. Thus in *Acetodextra amiuri*, the genital pore is not closely associated with the gonotyl; in *Allacanthochoasmus*, the pore is at

the base of the gonotyl; in *Cryptogonimus*, the genital atrium pierces the base of the gonotyl; while in other genera, the genital pore is at the extreme tip of the gonotyl. Since a true cirrus is lacking in these trematodes, the gonotyl probably functions as a cirrus or accessory copulatory structure, aided by the muscular ventrogenital cavity. Incidentally, there is a striking resemblance between the powerful muscles radiating from the ventrogenital pit in *Siphodera* and *Cryptocotyle*.

Knowledge of the life histories of *Exorchis oviformis*, *Caecinicola parvulus* and *Siphodera vinalledwardsii* indicate a close relationship between these and similar trematodes which Price (1940) has allocated to the family CRYPTOAGONIMIDAE. In known life histories, the cercariae are all of the pleurolophocercous type with an excretory formula of $2[(2+2) + (2+2)]$. The cercaria described by Hopkins (1938) also is probably a larval cryptogonimid; although its excretory pattern was not determined, it agrees with the cercariae of *Caecinicola* and *Siphodera* in possessing seven pairs of cephalic glands and a bilobed ventral caudal fin.

Both *Cercaria coronanda* Rothschild, 1938, and the cercaria of *Centrocestus armatus*, as described by Yamaguti (1938), have an excretory formula of $2[(2+2) + (2+2)]$ but differ in other respects from the cryptogonimid cercariae mentioned above. *C. coronanda* has ten pairs of cephalic glands with ducts in four bundles and the ventral caudal fin is not bilobed, while in the cercaria of *C. armatus*, there are only four pairs of cephalic glands with two bundles of ducts figured and caudal fins are completely lacking. Disparity in respect to the extent of caudal fins and number of cephalic glands may be correlated with the degree to which a species depends upon swimming and active penetration in establishing itself in the second intermediate host. Ingles (1935) has described a pleurolophocercous species which exists in the redia without escaping from the snail. In that species, a dorsal caudal fin is lacking and cephalic glands are neither figured nor described.

On the other hand, the identical excretory formulae in the cercarial stage of members of the families HETEROPHYIDAE, ACANTHOSTOMIDAE, and CRYPTOAGONIMIDAE raises a question concerning the separation of these families as proposed by Price (1940). In our opinion, most if not all of the characters on which the separation is based are of doubtful validity. Certainly body shape and size and the presence of eye-spot remnants in adults depend on the nature and extent of post-cercarial growth; in large species, the eye-spot pigment, if present in the cercaria, may become completely absorbed or so finely dispersed that it is easily overlooked, while in small species, it usually remains distinct. In all three families, circumoral spines are present in some genera and absent in others. Gonotyls are present in members of the HETEROPHYIDAE, ap-

parently absent in the ACANTHOSTOMIDAE, and either present or absent in the CRYPTOgonIMIDAE. In other papers (Cable and Hunninen, 1940, 1942) we have expressed the view that modifications of the terminal genitalia are by themselves of doubtful significance as characters of certain families. The opcoelid genera, *Podocotyle* and *Opecoeloides*, provide a striking example in support of this interpretation. Their cercariae are so similar that they can be differentiated only with difficulty and yet *Podocotyle* has a prominent cirrus sac and no accessory sucker while *Opecoeloides* has no cirrus sac but possesses an accessory sucker between the ventral sucker and the genital pore. In the family CRYPTOgonIMIDAE alone, variations and gradations in the development of the terminal genitalia-ventral sucker complex indicate that this complex is not a reliable character for differentiating this family from the ACANTHOSTOMIDAE and HETEROPHYIDAE.

While there is an exact agreement in the known excretory patterns of cryptogonimids, members of the family HETEROPHYIDAE show considerable variation in this respect. The pattern in *Cryptocotyle* is complex but in the remaining known excretory patterns of heterophyids, there are four groups of two or three flame cells on each side of the body, two groups joining each anterior and posterior collecting tubule. If *Centrocestus*, with an excretory formula identical to that of the cryptogonimids, is properly allocated to the family HETEROPHYIDAE, the excretory pattern does not afford a valid basis for separating that family from the ACANTHOSTOMIDAE and CRYPTOgonIMIDAE. Price's (1940) subdivision of the OPISTHORCHIOIDEA is based chiefly on the shape of the excretory vesicle. In the HETEROPHYIDAE, the vesicle is triangular or Y-shaped with short arms not extending anterior to the ovary while in the ACANTHOSTOMIDAE and CRYPTOgonIMIDAE, the vesicle is V- or Y-shaped with arms reaching the pharyngeal level. It should be stated that in heterophyids with Y-shaped vesicles, the ascending tubules join the tips of the arms while in *Siphodera* and probably other cryptogonimids and the ACANTHOSTOMIDAE, the tubules enter the arms at a considerable distance from their anterior ends. Since there seems to be no distinctly intermediate forms, these differences may justify the separation of the family HETEROPHYIDAE from the ACANTHOSTOMIDAE and CRYPTOgonIMIDAE. It should be pointed out, however, that in the cercarial stage, there is little if any indication of the shape or extent to which the excretory vesicle will develop in the adult worms.

The excretory vesicle of *Siphodera* is distinctly Y-shaped with a long stem and thus agrees with that of the ACANTHOSTOMIDAE as defined by Price (1940). Furthermore, certain of the genera allocated to the CRYPTOgonIMIDAE agree with the ACANTHOSTOMIDAE in lacking a gonotyl, although the metacercaria of *Cercaria coronanda*, presumably an

acanthostomid, possesses a distinct gonotyl. The chief difference, then, between the ACANTHOSTOMIDAE and CRYPTOgonimidae appears to be body shape. Although there may be significant differences in the arrangement of internal organs, many of these differences may be correlated with body size and shape. Therefore, it seems to us very doubtful that the ACANTHOSTOMIDAE and CRYPTOgonimidae should be retained as distinct families. The adult of *C. coronanda* was not determined by Rothschild (1938) although she later (1940) expressed the view that it probably would be between the Heterophyidae (Neochasminae) and ACANTHOSTOMIDAE but closer to the latter. Since Price (1940) has placed the NEOCHASMINAE in the CRYPTOgonimidae, it is quite likely that *C. coronanda* is a larval cryptogonimid. In view of that possibility, final disposition of the families ACANTHOSTOMIDAE and CRYPTOgonimidae must await knowledge of acanthostomid life histories, none of which to our knowledge has been traced experimentally.

SUMMARY

The life history of *Siphodera vinaledwardsii* (Linton, 1901) has been traced experimentally and various stages in the cycle are described. The cercaria is a pleurolophocercous form developing in rediae in the marine snail, *Bittium alternatum*. Fishes, particularly *Paralichthys dentatus*, serve as the second intermediate host. Metacercariae in experimentally infected flounders were fed to three toadfish, *Opsanus tau*, all of which were found at autopsy to harbor young adults of *S. vinaledwardsii*.

In a discussion of the opisthorchioid trematodes, it is concluded that the separation of the ACANTHOSTOMIDAE and CRYPTOgonimidae is of doubtful validity.

BIBLIOGRAPHY

- CABLE, R. M. AND HUNNINEN, A. V. 1940 Studies on the life history of *Spelotrema nicolli* (Trematoda: Microphallidae) with the description of a new microphallid cercaria. Biol. Bull. 78: 136-157.
- AND — 1942 Studies on *Deropristis inflata* (Molin), its life history and affinities to trematodes of the family Acanthocolpidae. Ibid. 82: 292-312.
- HOPKINS, S. H. 1938 A new heterophyid cercaria from Texas. J. Parasitol. 24 Suppl.: 26.
- INGLES, L. G. 1935 Notes on the development of a heterophyid trematode. Tr. Am. Micr. Soc. 54: 19-20.
- KOMIYA, Y. AND TAJIMI, T. 1940 Study on *Clonorchis sinensis* in the district of Shanghai. 6. The life cycle of *Exorchis oviformis* with special reference of the similarity of its larval forms to that of *Clonorchis sinensis*. J. Shanghai Sc. Inst. Sec. 4, 5: 109-123.
- LINTON, E. 1901 Parasites of fishes of the Woods Hole region. Bull. U. S. Fish Comm. (1899) 19: 405-492.
- 1905 Parasites of fishes of Beaufort, North Carolina. Bull. Bur. Fish. (1904) 24: 321-428.
- 1907 Notes on parasites of Bermuda fishes. Proc. U. S. Nat. Mus. 33: 85-126.

- 1910 Helminth fauna of the Dry Tortugas II. Trematodes. Carnegie Inst. Washington Publ. 133: 11-98.
- LUNDAHL, W. S. 1939 Life history of *Caecinicola parvulus* Marshall and Gilbert (Trematoda: Heterophyidae). J. Parasitol. 25 Suppl.: 27-28.
- 1941 Life history of *Caecinicola parvulus* Marshall and Gilbert (Cryptogonimidae, Trematoda) and the development of its excretory system. Tr. Am. Micr. Soc. 60: 461-484.
- MANTER, H. W. 1926 Some North American fish trematodes. Illinois Biol. Monogr. 10 (2), 138 pp.
- 1934 Some digenetic trematodes from deep-water fishes of Tortugas Florida. Carnegie Inst. Washington Publ. 435: 257-345.
- MUELLER, J. F. 1934 Parasites of Oneida Lake fishes. Part IV. Additional notes on parasites of Oneida Lake fishes including descriptions of new species. Roosevelt Wild Life Ann. 3: 335-373.
- PRICE, E. W. 1940 A review of the trematode superfamily Opisthorchioidea. Proc. Helm. Soc. Washington 7: 1-13.
- ROTHSCHILD, M. 1938 The excretory system of *Cercaria coronanda* n. sp. together with notes on its life history and the classification of cercariae of the superfamily Opisthorchioidea Vogel 1934 (Trematoda). Novit. Zool. 41: 148-163.
- 1940 A note on the systematic position of *Cercaria coronanda* Rothschild, 1938. Proc. Helm. Soc. Washington 7: 13-14.
- WILHELM, R. W. 1940 Serological reactions and species specificity of some helminths. Biol. Bull. 79: 64-90.
- WITENBERG, G. 1929 Studies on the trematode-family Heterophyidae. Ann. Trop. Med. and Parasitol. 23: 131-239.
- YAMAGUTI, S. 1938 Zur Entwicklungsgeschichte von *Centrocestus armatus* (Tanabe) mit besonderer Berücksichtigung der Cercarie. Z. Parasitenk. 10: 293-296.

EXPLANATION OF PLATE I

All figures pertain to *Siphodera vinaledwardsii*.

ABBREVIATIONS

DG dermal glands	OT ootype
ED ejaculatory duct	OV ovary
EP excretory pore	PG prostate glands
ES esophagus	PH pharynx
EV excretory vesicle	PP prepharynx
EY eye-spot remnants	PR prostatic bulb
GL glands around ventrogenital pit	SR seminal receptacle
GP genital pore	SV ₁ seminal vesicle, terminal portion
GS genital sinus or atrium	SV ₂ seminal vesicle, beginning portion
IN intestine	UT uterus
LC Laurer's canal	VA vagina or metraterm
MG Mehlis' gland	VD transverse vitelline duct
MO mouth	VG ventrogenital pit
MU muscle	VI vitellaria
OD oviduct	VR vitelline reservoir
OS oral sucker	VS ventral sucker

FIG. 1. Adult, dorsal view.

FIG. 2. Adult, slightly oblique sagittal section.

FIG. 3. Adult, cross-section at level of ventral sucker.

FIG. 4. Free-hand drawing of spines in ventrogenital pit as observed in living adult worm.

FIG. 5. Free-hand drawing of reproductive system as observed in living adults under considerable coverglass pressure.

FIG. 6. Eggs.

FIG. 7. Adult, excretory system (free-hand).

EXPLANATION OF PLATE II, p. 422

All figures pertain to *Siphodera vinaledwardsii*.

ABBREVIATIONS

CG cephalic glands	GB germ balls
DF dorsal fin-fold of tail	GD cephalic gland ducts
EP excretory pore	IN intestine
EV excretory vesicle	OG openings of cephalic glands
EY eye-spot	PH pharynx
GA genital primordium	VF ventral fin-fold of tail
	VS ventral sucker

FIG. 8. Redia showing excretory system of one side.

FIG. 9. Cercaria, ventral view.

FIG. 10. Free-hand drawing showing resting position of cercaria.

FIG. 11. Metacercariae (a) 24 hours, (b) 48 hours, and (c) 7 days of age.

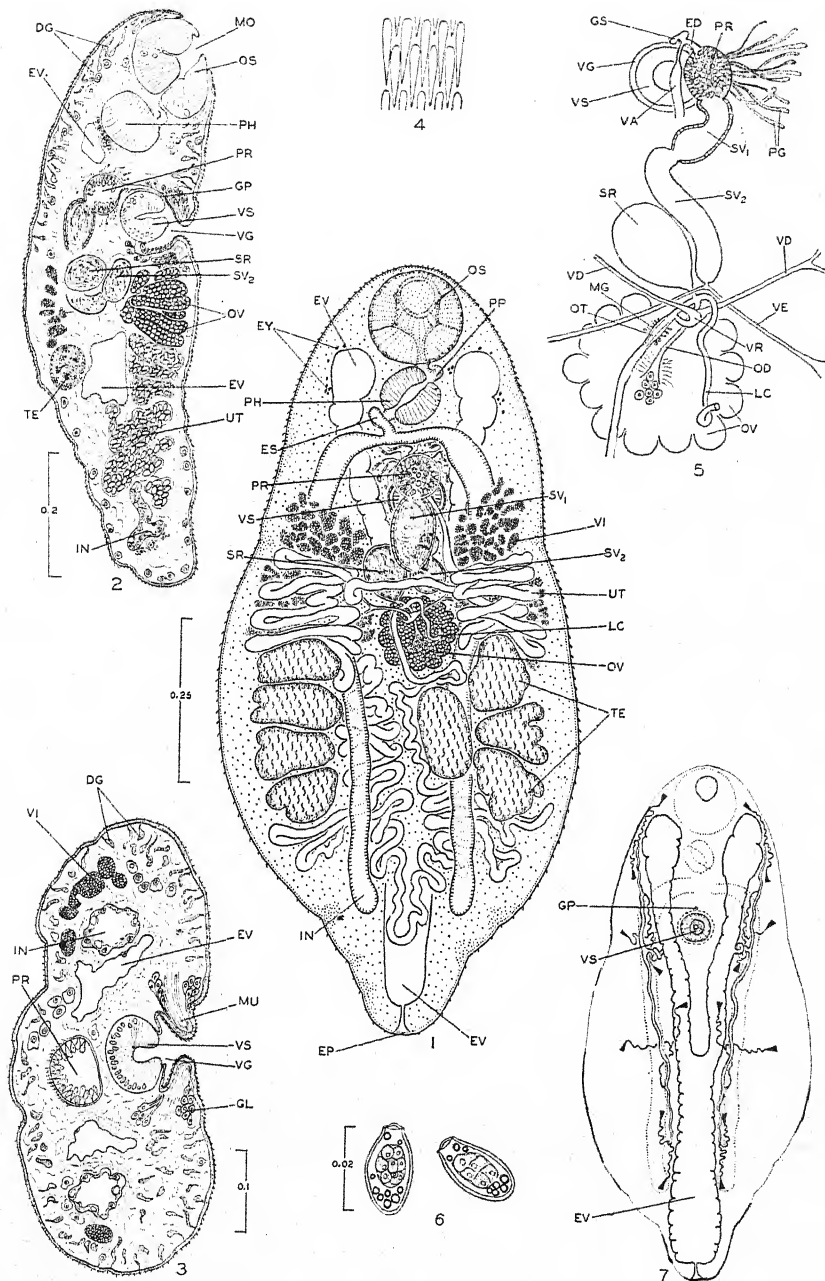


PLATE I

RESEARCH NOTES

PARASITES OF THE WOODLAND CARIBOU

Dikmans (1938, Proc. Helm. Soc. Washington 6: 97-101) had for his review of the parasites of semi-domesticated and wild ruminants no information on the helminths of the woodland caribou. Since that time, however, three of these hosts, *Rangifer caribou sylvestris*, have been examined for parasites in our laboratory at University Farm and one has been examined in the field in Canada and the helminths recovered from it submitted to us for identification.

Three of the four animals examined were captured by Mr. J. Manweiler at Montreal Lake, Saskatchewan in 1938. The first one, an adult female, was trapped on May 9, 1938. It died and was examined for parasites in the field at Montreal Lake by Mr. Manweiler. Several larval specimens of the tapeworm, *Taenia krabbei*, were removed from the muscles of its right shoulder, and 5 nematodes, *Dictyocaulus viviparus*, were taken from its lungs. It was also parasitized by 66 nose bots, *Cephenomyia phobifer*, 3 of which Mr. Manweiler reared. All of these parasites, with the exception of the 3 bots reared by Mr. Manweiler, were submitted to us for identification and have been placed in the helminthological collection of the Division of Entomology and Economic Zoology, University of Minnesota.

The second caribou, a month old calf, was captured on May 25, 1938. Only the viscera of the calf were available for study; no parasites were found.

The third animal, a yearling, was shipped from Montreal Lake, Saskatchewan, to the caribou refuge at Red Lake, Minnesota, where it lived for 3 years, dying on December 28, 1940. At autopsy 16 specimens of the filarial nematode, *Setaria cervi* [= *labiatopapillosa*] were found attached to the outside wall of its stomach.

The fourth caribou, an old female and one of the very few survivors of the native herd at Red Lake, Minnesota, which is the only region of the state where native caribou still survive, was found in a weakened condition by Mr. Manweiler about August 10, 1940. It was sent to University Farm for observation and then removed to the Carolos Avery Game Refuge in Anoka County where it died on August 18, 1940, probably due to the infirmities of advanced age. Three species of helminths were recovered from it: 15 lung worms, *Dictyocaulus viviparus*, 2 filarial worms, *Setaria cervi*, and 5 tapeworms, *Moniesia expansa*. In addition, Dr. R. Fenstermacher noted old liver abscesses, probably caused by the fluke *Fascioloides magna*. No flukes were found, but a few eggs were noted.

We are indebted to Mr. Allen McIntosh of the Zoological Division, Bureau of Animal Industry who made the specific identification of the tapeworms, and to Dr. G. Dikmans and Mr. John T. Lucker who verified our identifications of the nematodes. Also, we wish to thank Mr. Manweiler who spared no effort in getting the caribou to us.—ARNOLD B. ERICKSON, *Division of Economic Zoology, University of Minnesota*, AND P. R. HIGHBY, *Minnesota Conservation Department, Division of Game and Fish*.

A NOTE ON IMMUNITY REACTION IN THE BLACK-HEADED GULL (*LARUS RIDIBUNDUS* L.) INFECTED WITH *MARITREMA OÖCYSTA* LEBOUR, 1907

Very numerous American observers have drawn attention to the fact that: (1) very heavy infections of trematodes are not long supported by the host, which passes out numbers of mature and immature worms in the feces until the infection is greatly reduced; (2) reinfection is often unsuccessful, some type of immunity being set up.

During my work on the life-cycle of *Maritrema oöcysta* Lebour, 1907 a similar phenomenon was noted. One observation was made which seems worth recording.

Twenty thousand encysted cercariae were fed to a laboratory-reared black-

headed gull (*Larus ridibundus* L.) every day for eight days. The peak of the infection appeared to be reached about the sixth day, when vast numbers of viable eggs (which hatched on the third day), but no worms, were observed in the feces. From that date onwards the infection began to decline very rapidly, moribund worms in all stages of development being passed out, and the egg count fell precipitately. At the same time it became evident that large numbers of unexcysted worms were also appearing in the feces. At first it was thought that these were another closely allied species, accidentally fed to the bird, and were not developing in this particular host. This mistake can be made very easily, owing to the remarkable similarity between metacercariae of closely related species of *Maritrema*. I concluded, however, that they were in fact *M. oöcysta* cysts. Two possible explanations occurred to me. (1) Although the bird did not appear to be suffering from any intestinal disturbance due to the infection, as a result of the irritation caused by the worms, increased peristalsis passed the cysts so rapidly down the intestine that they avoided the action of localized enzymes normally responsible for the break-down of the cyst-wall. (2) That the production of the enzymes in question was greatly reduced or actually inhibited.

The cyst-wall of *M. oöcysta* is, however, remarkably susceptible to the action of enzymes, and artificial excystment can always be induced in the species with unusual ease. With these facts in mind ten days later I fed this gull several specimens of *Scrobicularia* sp., which I had experimentally infected with a few echinostome cercariae also obtained from *Peringia ulvae* Pennant, 1777. Ten days later several echinostome eggs, but no *Maritrema* eggs, were found in the feces. Forty thousand *M. oöcysta* cysts were again fed to the gull on three consecutive days. Subsequently fecal examinations revealed only an occasional *Maritrema* egg and a few echinostome eggs. Unfortunately enemy action terminated this experiment prematurely. It was intended to keep the bird alive for a considerable period, in order to discover how long an infection will last once it has become stabilized. This particular gull was killed by the effect of blast from a high explosive bomb, and the examination was consequently made only three months after the initial infection with *M. oöcysta*. When an infection of this worm is at its height, it is fairly well distributed along the entire length of the intestine, whereas in a stabilized infection the worms are confined to the terminal portion. In this particular bird only one worm was found in the upper end of the intestine, and the remaining specimens were all situated in the rectum.

It would therefore appear that in black-headed gull very heavy infection with *M. oöcysta* sets up an immunity reaction, which limits the number of the parasites, and to a certain degree prohibits the excystment of the metacercariae. On the other hand, this does not preclude reinfection with another species of trematode. —MIRIAM ROTHSCHILD, Ashton Wold, Peterborough, England.

ADELINA DERONIS N. SP., A NEW COCCIDIAN PARASITE OF
THE AQUATIC OLIGOCHAETE, *DERO LIMOSA*

This parasite was discovered in the coelomic cavities of living specimens of the naid oligochaete, *Dero limosa*. The worms were from a small pond on the university campus. Examination of numerous specimens from 8 other naid genera collected in Pennsylvania, New Jersey and the New England states has not revealed infections of *Adelina deronis* or any other sporozoan. This apparent host-specificity justifies the name chosen for the new parasite.

Adelina deronis n. sp.

(Figs. 1-5)

Sporozoites: 12 microns long, 2.5 microns wide. Ovoid nucleus in posterior third.

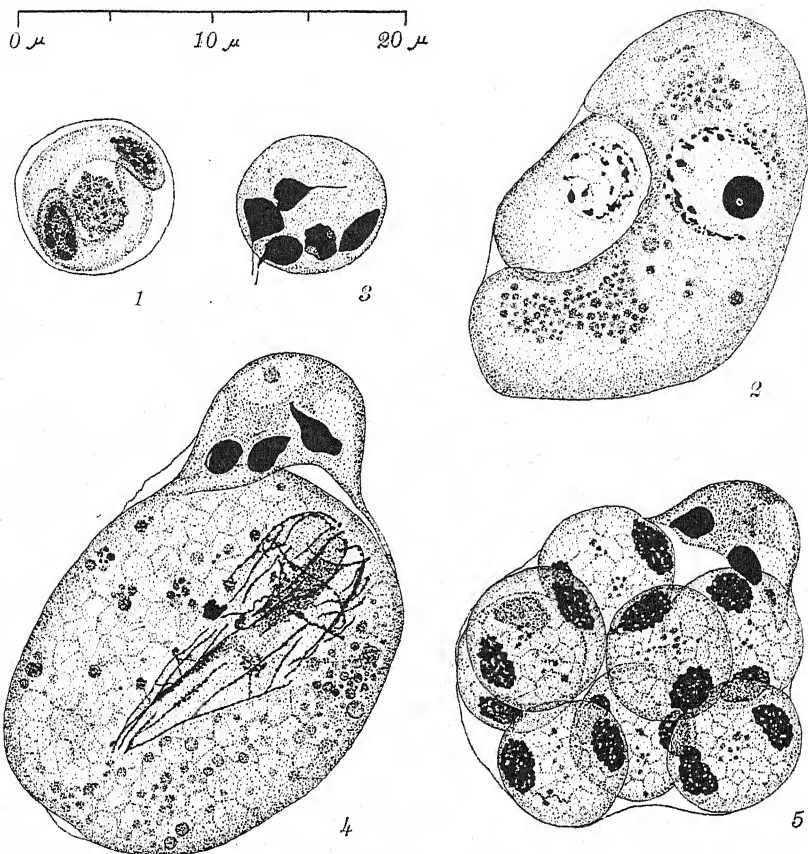
Trophozoites (Schizonts): Spherical to ovoid. Mature stages 25 microns in diameter with up to 60 nuclei.

Merozoites: From 6 to 13 microns long, and 1.5 to 3 microns wide, depending on generation.

Microgametocytes: Sub-spherical, and from 7 to 9 microns in diameter. Each produces four flagellated microgametes, 2 microns long, 1.5 microns wide.

Macrogametocytes: Ovoid, averaging 19 microns in length, 17 microns in width.

Oöcysts: 17 to 21 microns in diameter, thin-walled and slightly lobed; 8 to 16 sporocysts.



Adelina deconis n. sp.

FIG. 1. Mature sporocyst containing two sporozoites and a central residual body.

FIG. 2. Advanced stage of gametocyte association. Pair is held together by copulation membrane. Nucleus of the (smaller) microgametocyte in early prophase. Conspicuous nucleolus and peripheral chromatin in macrogametocyte nucleus.

FIG. 3. Mature microgametocyte (drawn without the attached female) containing four flagellated microgametes and one nucleolar remnant.

FIG. 4. Zygote with attached microgametocyte remnant. Synkaryon spindle shows maternal chromatin (upper half) and paternal chromatin (lower half). Fertilization-membrane is slightly raised at lower pole, where microgamete has entered. Three unused microgametes left behind in the microgametocyte.

FIG. 5. Advanced oöcyst containing eight immature sporocysts. Microgametocyte remnant still attached at upper right.

Sporocysts: Thin-walled, spherical, 6 to 7 microns in diameter. Contain 2 sporozoites and a centrally located, granular residual body.

Host: *Dero limosa*.

Site of Infection: Peritoneal tissue and coelomic cavities.

Locality: Pond on campus of University of Pennsylvania.

Studies of living and fixed material, smears as well as sections, have shown the following life-cycle. Sporocysts (Fig. 1) are presumably ingested by the worm, and emerging sporozoites probably penetrate through the midgut wall into the coelom. Here they enter the cells of the parietal peritoneum, where they become enlarged, round up, and undergo schizogony. From 20 to over 60 merozoites are produced by each schizont. The vegetative cycle shows at least two morphologically distinct generations. It is possible to distinguish between vegetative and gametocyte-producing schizonts. The latter are of two types; one gives rise to an average of 30 microgametocytes, the other to an average of 8 macrogametocytes. These gametocytes become associated at an early stage of development. A syzygy membrane is formed around each pair (Fig. 2). After a period of growth, the microgametocyte gives rise to 4 flagellated microgametes (Fig. 3), one of which enters the female cell at a point opposite the region of original union of the gametocytes. Fertilization is followed by the secretion of a fertilization membrane. The maternal and paternal chromatin becomes arranged end to end in the synkaryon spindle (Fig. 4). This now undergoes condensation, and usually withdraws toward the pole nearest the microgametocyte remnant. The zygote nucleus is the only diploid nucleus in the entire cycle. The haploid chromosome number is established at the first zygotic division. The zygote (oöcyst), to which microgametocyte remnants are usually still attached (Fig. 5), produces 8 to 16 sporocysts, each with two sporozoites (cf. Fig. 1).

The mature oöcyst is the only stage suitable for species determination. *A. deronis* cysts are distinct from those of *A. octospora* Hesse (Sur le genre *Adelea* à propos d'une nouvelle coccidie des oligochètes. Arch. Zool. Exp., VII, XV, 1911), the only species with which they might be confused. The oöcysts of *A. octospora* are smoothly spherical, thick-walled, and 19 to 23 microns in diameter. They invariably produce 8 spherical, thick-walled sporocysts, 9 microns in diameter and containing 2 sporozoites each, in contrast to the characteristics given above for the new species.

A detailed cytological account of *A. deronis* is being prepared for later publication.—T. S. HAUSCHKA AND M. I. PENNYPACKER, *Zoological Laboratory, University of Pennsylvania*.

EXCYSTATION OF COCCIDIAL OÖCYSTS OF THE CHICKEN

In a paper by Pratt (1937, J. Parasitol. 23: 426-427), it was reported that spores and sporozoites of the chicken coccidium, *Eimeria tenella*, were liberated in the crop of birds, in some cases as soon as five minutes after the oöcysts were ingested. His attempts to excyst the oöcysts in vitro with pancreatin, and with saline extracts of the crop, proventriculus, and duodenum failed. Although no positive statement was made, his results strongly inferred that digestive enzymes were not responsible for excystation. This is contrary to the findings of others, particularly Smetana (1933, Arch. Path. 15: 175-192) who demonstrated, by well-controlled in vitro experiments, that trypsin was responsible for excystation of rabbit coccidia. This difference of opinion, in addition to the scarcity of definitive information prompted the writer to investigate the problem.

The pancreatic ducts were ligated in a number of coccidia-free White Leghorn chickens from five to twelve weeks of age. The ligated birds and normal controls were dosed into the crop with equal amounts of uniform suspensions of sporulated oöcysts. Fecal examinations for the resultant oöcyst discharge were made at suitable intervals by means of the sugar flotation technique. Negative results were checked further by the more sensitive test of feeding the incubated feces to coccidia-free birds. Ten gram samples of the feces in question were incubated for three days

at 30° C in shallow layers of 1½ per cent potassium dichromate solution. The incubated feces were then fed to coccidia-free birds and their feces, in turn, were examined for resultant infection in the usual manner. The objection might be raised that the intestinal epithelium of the ligated birds might be so altered physiologically that growth of the parasites could not be supported in any case. To answer this objection, infection of the ligated birds was attempted with merozoites (Levine, 1940, J. Parasitol. 26: 337-343).

The results of these experiments (Table 1) were consistent. None of the birds with ligated pancreatic ducts became infected when dosed with sporulated oöcysts.

TABLE 1.—*Coccidial infection of normal chickens and chickens with ligated pancreatic ducts dosed with sporulated oöcysts and with merozoites. Infection determined by fecal examination for oöcysts (sugar flotation technique) and by feeding tests with incubated feces*

		Days after feeding sporulated oöcysts																
		4	5	6	7	8	9	10	11	12	13	15	17	19	27	29		
<i>E. praecox</i>	Ligated	-	-	-														
	Control	+	+	+									m3	+				
<i>E. mitis</i>	Ligated	-	-	-	-			m3		+								
	Control		+	+	+													
<i>E. maxima</i>	Ligated			-	-	-	-		m4		+							
	Control			+	+	+												
<i>E. hagani</i>	Ligated				-	-	-											
	Control				+	+	+											
<i>E. tenella</i>	Ligated				-	-	-		-									
	Control				+	+	+								m5	+		
<i>E. necatrix</i>	Ligated				-	-	-											
	Control				+	+	+				m5	+						

M3, m4, m5 = Merozoites (collected from a bird destroyed on the day of infection indicated by the number) injected into the intestine or into the ceca (*E. tenella* experiment).

This is apparent from the negative findings of fecal examinations. Feeding tests with the incubated feces were likewise negative. In the experiments with *E. tenella* and *E. necatrix* the control birds exhibited symptoms and post mortem lesions characteristic of infection with those species. The ligated birds, however, remained normal in every respect. On the other hand, in every instance where merozoites were administered, infection resulted promptly. This observation indicated that the intestinal epithelium of ligated birds was fully capable of supporting coccidial growth. Precautions had to be taken in ligating the pancreatic ducts, since imperfect ligation permitting even a small flow of pancreatic juice, rendered the bird susceptible to infection with sporulated oöcysts. In one instance where the ducts were not only ligated but also severed, slight infection resulted from a dose of sporulated oöcysts. This unexpected result was explained when the bird was examined post mortem. It was found that in addition to the three pancreatic ducts normally present in the chicken there was a fourth duct originating from the posterior end of the pancreas discharging secretions into the duodenum at the curve of the U. This anomaly had been overlooked when the other ducts had been ligated.

Since sporulated oöcysts had failed to infect ligated birds while merozoites were successful, it seemed logical to assume that excystation of the oöcysts had failed to take place. In that case the sporulated oöcysts should have passed through the intestinal tract unchanged. To test this hypothesis all the feces passed by the ligated and control birds in the experiment with *E. praecox* were collected during the 24 hours following the dosing with the sporulated oöcysts. The feces were stored in a refrigerator for 24 hours and then fed to each of two coccidia-free chickens. The relative number of sporulated oöcysts in each lot of feces was estimated by comparing the number of oöcysts discharged by these chickens. It was estimated that the

feces of the ligated bird produced 100 times as many oöcysts as those of the control. A similar experiment was done in the test with *E. tenella*. Again the chicken that received the feces of the ligated birds suffered an infection which was much more severe than the one occurring in the bird fed the feces of the control. This was evidenced by a comparison not only of the relative number of oöcysts discharged by each but also by the severity of the cecal lesions.

In the writer's opinion the only reasonable interpretation of the results herein described is that pancreatic juice is necessary for the excystation of oöcysts of the chicken. Some preliminary experiments in vitro done by the author indicate that trypsin may be the active agent as was found by Smetana with rabbit coccidia. Pratt's findings may possibly be explained either by assuming that a regurgitation of enzyme-containing fluid took place in the crop or that sporozoites were released in that organ as a result of mechanical pressure. In any event our data indicate that sporozoites, if liberated in that organ, could not produce infection.—P. P. LEVINE, *New York State Veterinary College, Ithaca, New York*.

A NEW THERMOSTABLE MEDIUM FOR THE PROLONGED BACTERIA-FREE CULTIVATION OF *TRICHOMONAS* *FOETUS*

A variety of media have been successfully employed for cultivation of bacteria-free cultures of *Trichomonas foetus*, including those of Glaser and Coria (1935, *Am. J. Hyg.* 22: 221-226), Rees (1937, *Am. J. Hyg.* 26: 283-291) and Daniel (1940, *J. Parasitol.* 26: 85). It was found these would support growth sufficient to maintain cultures over an indefinite period of time, provided frequent transfers were made. Nevertheless, with the exception of Andrews and Lyford (1940, *Am. J. Hyg.* 31C: 43-50) and recently of Lyford (1941, *Am. J. Hyg.* 33C: 69-87), who were able to maintain individual cultures for as long as 3-4 months, none of the media developed support growth of individual cultures for long intervals (rarely longer than 3 weeks) and yet make subculturing possible.

The following notes are upon a thermostable sodium citrate medium originated by Schneider (1941, Thesis, Univ. Wisconsin Library), and later modified by the addition of dried ground egg shell (1942, Schneider, *J. Vet. Res.*, in press). A sufficient amount of this medium can be prepared in one day to last, when kept at ice-box temperature, for a number of weeks, with no resultant effect on the growth of *Trichomonas foetus*. It has supported the growth of two strains of *T. foetus*, one of which has been maintained on it for nearly two years, the other since its isolation on March 31, 1941.

At 37° C individual cultures, covered with "Parafilm" or in anaerobic glass-sealed test tubes (as used for transportation purposes), have lasted 30-60 days, with the longest on record 72 days. At room temperature, 22-25° C, such cultures have been kept 3-4 months. The longest positive cultures recorded were 139 and 147 days old, respectively. Subcultures were easily started from them. After prolonged storing at room temperature, cultures are placed in a 37° C incubator for 3 to 10 days to permit the flagellates to multiply in sufficient numbers (as noted by the development of gas bubbles at the surface of the liquid portion of the medium) to allow for making subinoculations. The medium is recommended for maintaining stock cultures of *T. foetus* at room temperature and is prepared in two parts.

A. Preparation of egg slants:

6 whole eggs

60 ml defibrinated bovine blood

50 ml of "Sodium Carbonate Solution" prepared as follows:

Sodium Chloride	6.5 gm
Sodium Bicarbonate	0.2 gm
Potassium Chloride	0.2 gm
Sodium Carbonate, Anhydrous, ($\text{Na}_2\text{CO}_3 + 10 \text{H}_2\text{O}$ can be substituted)	0.5 gm

Potassium Phosphate, Monobasic (KH_2PO_4)	0.5 gm
Potassium Phosphate, Dibasic (K_2HPO_4)	0.5 gm
Glucose	2.5 gm
Distilled water	1000 ml

1. Beat up thoroughly with glass beads in a two-liter flask.
2. Filter through cheese cloth or gauze to remove membranes, etc.
3. Distribute in test tubes in 4 to 5 ml quantities.
4. Slant test tubes in previously heated autoclave, turn on steam till pressure reaches 10-12 pounds, and then turn steam off completely. Remove immediately when cool. It is to be noted that this does not sterilize the egg slants.

B. Preparation of liquid portion of the medium for covering the egg slants:

1. Sodium Citrate Solution	
Sodium Chloride	5.0 gm
Magnesium Sulphate	0.2 gm
Ammonium Acid Phosphate ($\text{NH}_4\text{H}_2\text{PO}_4$)	1.0 gm
Potassium Phosphate, Dibasic (K_2HPO_4)	1.0 gm
Sodium Citrate	2.0 gm
Glucose	10.0 gm
Distilled water	1000 ml

2. Bovine serum (sterility not required) 5.0-7.5%. The serum is always centrifuged two to three times to remove all cellular material.
3. Adjust pH to 7.2-7.4.
4. Add 2 ml of a 1.6% alcoholic solution of bromcresolpurple (for visibly observing pH change, indicating flagellate growth in medium).
5. Add 40 mgm hematein (dissolved in distilled water so that each ml contains 5 mgm hematein. Ten minutes in the autoclave at 15 pounds pressure will help the hematein to go into solution).
6. Filter two to three times through double layer filter paper until the solution is perfectly clear.
7. Add dried ground egg shell on the bottom of the egg slant to a height of about 5-10 mm.
8. 10-12 ml of the filtered solution (direction #6) is used to cover the egg slant and dried egg shell.
9. Sterilize in autoclave 30 minutes, 15 pounds pressure.

MORRIS D. SCHNEIDER, *St. Paul, Minnesota.*

SOUTH AMERICAN CUTANEOUS LEISHMANIASIS IN EXPERIMENTAL ANIMALS

Under the name leishmaniasis are included the human diseases caused by protozoa belonging to the genus *Leishmania*. The clinical forms of the disease may be classified under at least two groups, visceral and cutaneous. In the cutaneous disease, which occurs in South and Central America, India, Asia and Africa, single or multiple ulcerating granulomatous lesions are found on exposed parts of the body, and no invasion of the viscera is reported. In the American form of cutaneous leishmaniasis, the mucous membranes of the nasopharynx may also be affected in 10 to 50 per cent of cases in particular localities.

The etiological agent of South American cutaneous leishmaniasis, named *Leishmania brasiliensis* Vianna (1911, Brazil-Med. 25: 411), may be recovered from the skin lesions in smears and cultures under favorable conditions. Isolation is frequently made difficult by reason of secondary bacterial infection of many lesions.

Experimental attempts to produce the disease in many different animals have been attended with varying degrees of success (Geiman, 1940, J. Parasitol. 26 Suppl.: 22-23). A fundamental problem has been to find an animal that is uniformly susceptible to cutaneous infection, thereby enabling one to carry out critical studies

of transmission, immunology, and comparative pathology of the disease. In the present investigation animals have been used which are closely related to species known to be susceptible to visceral leishmaniasis. Thus we have studied Syrian hamsters, *Cricetus auratus*, Texas ground squirrels, *Citellus tridecemlineatus* subspecies, and a few of the members of the Sciuridae indigenous to the eastern United States.

The organisms used for inoculation of animals were isolated in 1939 in Peru from human cases of cutaneous leishmaniasis (Geiman, 1940). They have been maintained subsequently through serial passage on the well-known NNN medium of Novy, MacNeal and Nicolle (Nicolle, 1908, Bull. Soc. Path. Exot. 1: 121-126). Material obtained from such cultures, suspended in normal saline, has served for inoculation of animals by subcutaneous or intraperitoneal routes, or combinations of both. After inoculation, an animal is observed at regular intervals for signs of disease. If a cutaneous lesion appears, it is punctured under aseptic precautions to demonstrate and recover organisms. Some of the material thus obtained is planted on NNN medium, and the culture is examined for flagellates until it is a month old. Smears of material obtained by puncture are fixed in absolute methyl alcohol, stained with Giemsa solution, and examined for cells containing parasites in the form of typical Leishman-Donovan bodies. An animal is declared to be positive only after it has developed a gross lesion from which characteristic forms of the parasite have been recovered in culture or in smears. Biopsy specimens have been taken from certain lesions from time to time for implantation into other animals and for sectioning. When an animal is autopsied, routine smears and cultures are made of heart's blood, spleen, liver, and bone marrow.

This study of three strains of *Leishmania brasiliensis* has given the following results:

Syrian hamsters have shown no evidence of infection following intraperitoneal injection of culture material. Fourteen animals have been injected subcutaneously. Only three of these have developed skin lesions, and they were inoculated with organisms which had been subjected to passage through chick embryos (Geiman, 1940). The lesions consisted of indurated nodules which were first noticed at the injection sites 55, 74, and 90 days after inoculation. The nodules gradually increased in size to a diameter of 5-7 mm. The overlying skin has remained intact, and the lesions have failed to ulcerate. The lesions have persisted in two of these animals for eleven months, at which time the parasites could still be recovered in smears and cultures. There has been no apparent effect on the general well-being of the animals. The third positive animal died of unknown cause eight months after inoculation, and, owing to an accident, no autopsy was performed.

Ten Texas ground squirrels have received series of four intraperitoneal injections of culture material at short intervals. No evidence of infection has been found in these ten animals during life or at autopsy. Five of these squirrels, subsequent to their series of intraperitoneal injections, received subcutaneous injections of the same strain with which they had been injected intraperitoneally, but no lesions developed. A sixth squirrel, however, was injected subcutaneously with a strain different from that used for intraperitoneal injection. A nodular cutaneous lesion was first noticed 97 days later at the site of injection. This has persisted as a small nodule during the ensuing eight months, with no signs of ulceration or regression, and organisms have been recovered at intervals.

Subcutaneous injections of culture material were given to eight normal Texas ground squirrels, and all eight animals developed nodular skin lesions after periods ranging from 21 to 44 days. In every case the organisms have been recovered in smears and cultures. The nodules have subsequently ulcerated, and have persisted in the latter form for more than 11 months in some cases. Serial passage to other ground squirrels and to hamsters has been effected, with production of cutaneous lesions. Thus Koch's postulates have been fulfilled.

A red squirrel, *Sciurus hudsonicus loquax*, was injected subcutaneously with culture material, but this animal died of unknown cause two weeks later.

Two gray squirrels, *Sciurus carolinensis leucotis*, and one chipmunk, *Tamias striatus lysteri*, have developed nodular skin lesions following subcutaneous injection. The two squirrels were killed and autopsied soon after the nodules had begun to develop. The lesion of the chipmunk regressed after three months without undergoing ulceration. The organisms were recovered from each animal.

The pathology of experimental South American cutaneous leishmaniasis in squirrels has been studied. It is similar to the reaction produced in human beings, consisting of an accumulation of mononuclear phagocytes containing the parasites. An indurated papule is formed after an incubation period of about three to eight weeks. This increases in size and may ulcerate. A notable difference, however, lies in the observation that the ulcerated lesions of the squirrels remain remarkably free from evidence of secondary bacterial invasion, which is quite common in the human skin lesions. Furthermore, we have not observed invasion of the nasopharynx in squirrels.

The results of the present study suggest the usefulness of ground squirrels in the investigation of South American cutaneous leishmaniasis. Further work is in progress at the present time, and special attention is being directed toward the pathology and immunology of the disease.—H. S. FULLER AND Q. M. GEIMAN, *Department of Comparative Pathology and Tropical Medicine, Schools of Medicine and Public Health, Harvard University.*

HELMINTHS FROM THE NORWAY RAT IN NORTHEASTERN OHIO

In the winter and spring of 1940-41 a survey was made of helminthic parasites harbored by Norway rats, *Rattus norvegicus*, in the vicinity of Kent, Portage County, in northeastern Ohio. From twelve different locations, including markets, homes, dumps, barns, slaughter houses, and warehouses, 50 rats were trapped alive and then chloroformed for examination. Identification was confirmed on preserved adult worms with the assistance of the keys of Yorke and Maplestone (1926, *Nematode Parasites of Vertebrates*) for the nematodes, and of Meggitt and Subramanian (1924, *Burma Res. Soc.* 17: 190-237) for the tapeworms.

Helminths were found in 49, or 98% of the rats examined, representing three species of cestodes and four of nematodes: *Hymenolepis nana* (Siebold, 1852) in 26%; *H. diminuta* (Rudolphi, 1808) in 28%; the larval form *Cysticercus fasciolaris* (Rudolphi, 1808) in 10%; *Trichosomoides crassicauda* (Bellingham, 1840) in 42%; *Nippostrongylus muris* (Yokogawa, 1920) in 4%; *Ganguleterakis spumosa* (Schneider, 1866) in 42%; and *Syphacia obvelata* (Rudolphi, 1802) in 4% of the rats examined.

The number of specimens per rat of *H. nana* varied from 1 to 12; of *H. diminuta* from 1 to 4; of *C. fasciolaris* 1 and 2; of *T. crassicauda* from 2 to 20, averaging 7.3; of *N. muris* 8 and 20; of *G. spumosa* from 1 to 58, averaging 15. For *G. spumosa* the ratio of males to females was 2.7:1.7; 57% of the specimens were in the colon, and 43% in the cecum, with one specimen aberrant in the kidney of one rat and one in the spleen of another. Of the two cases of *S. obvelata*, one rat had 58 females and 2 males, and the second rat two females only in the colon.

Multiple infections were common, one rat harboring five of the seven species found, lacking only *N. muris* and *S. obvelata*; in three rats four species were found, in two of which were *H. nana*, *H. diminuta*, *T. crassicauda*, and *G. spumosa*, and in the third, *H. nana*, *T. crassicauda*, *G. spumosa*, and *S. obvelata*; in four rats three species were found, in different combinations including the species found except *N. muris*; of twenty-one rats harboring two species, six had *H. diminuta* and *G. spumosa*, and five had *H. nana* and *T. crassicauda*; and in nineteen rats only one species was found, in nine of which it was *G. spumosa*.—WILLIAM C. FORBES, *Kent State University, Kent, Ohio.*

THE LIFE CYCLE OF THE TREMATODE *ECHINOSTOMUM* *CALLAWAYENSIS* BARKER

Considerable numbers of echinostome metacercariae, averaging 0.13 mm in diameter, were found encysted in specimens of *Planaria* being prepared for whole

mounts. When fed to laboratory rats these metacercariae developed in 13 days into mature trematodes identified as *Echinostomum callawayensis* Barker (1915, J. Parasitol. 1: 184-197), described originally from material obtained from the intestine of the muskrat. The only essential difference between Barker's specimens and those reared in rats was in the presence of fewer eggs in the latter.

The first intermediate host proved to be the snail *Physa gyrina*. Naturally infected snails, and also *Planaria*, were obtained from a lake in Ramsey County, Minnesota, where muskrats are abundant. Rediae were found to be of the echinostome type having a short gut and producing cercariae with two rows of about 45 spines. The body of the cercaria measures about 0.30 mm long by 0.18 mm wide, the suckers are subequal, 0.050 to 0.055 mm in diameter, and the tail is approximately 0.32 mm long.

Cercariae are shed in the evening and live for about 24 hours in laboratory containers. When they come in contact with *Planaria* they actively crawl over the surface of the animal, constantly trying the surface. Penetration requires several hours and was repeatedly brought about on uninfected hosts. When fed to rats the cysts produced mature flukes. Tadpoles and guppies could not be infected with the metacercariae.

Aid in the study of the metacercaria was given by Eugene Breault, a student. Details of the life cycle will be published in a subsequent paper.—RALPH W. MACY, *College of St. Thomas, St. Paul, Minnesota.*

AMERICAN SOCIETY OF PARASITOLOGISTS

PRELIMINARY ANNOUNCEMENT OF THE EIGHTEENTH ANNUAL MEETING

MONDAY, TUESDAY, AND WEDNESDAY, DECEMBER 28-30, 1942

NEW YORK, NEW YORK

In accordance with the action of the Council, the American Society of Parasitologists will convene for a three-day program in conjunction with the meeting of the American Association for the Advancement of Science in New York, New York. The Commodore Hotel has been designated as official headquarters of the Society. The regular meetings will be held in Hunter College, 68th Street and Park Avenue, New York.

The program will be arranged in the same manner as in previous years, with all of the first and third days devoted to contributed papers. The address of the retiring president, Dr. Henry E. Meleney on the subject, "The Role of Parasitologists in the Second World War," will be delivered at the conclusion of the morning session of the second day. The annual luncheon and business meeting will follow the presidential address, and the demonstration program and tea will be held in the afternoon. The demonstration-tea is planned to serve both as a scientific program and as a social function, and to supply the opportunity for informal discussion that is not possible at the regular scientific sessions.

The call for papers has already been sent to members of the Society. In order that the abstracts be published and mailed to the membership before the meetings, it is necessary that they be received in the office of the secretary not later than October 17, 1942. Members are hereby reminded of this date, and are requested to be prompt in the submission of papers for the program.

JAMES T. CULBERTSON, *Secretary*

NOTICE: NEW YORK MEETING POSTPONED

The Journal of Parasitology

Volume 28

December Supplement, 1942

PROGRAM AND ABSTRACTS OF THE EIGHTEENTH ANNUAL MEETING OF THE AMERICAN SOCIETY OF PARASITOLOGISTS

NEW YORK, NEW YORK

DECEMBER 28, 29, and 30, 1942

PROGRAM¹

MONDAY AFTERNOON SESSION, DECEMBER 28, 2:00 P.M.; Room 919,
HUNTER COLLEGE, 695 PARK AVENUE.

Read

1. Development of the Metacercariae of *Diplostomum flexicaudum* in the Lenses of Frogs, Turtles, Birds, and Mammals. (15 min) (Lantern) M. S. FERGUSON, Rockefeller Institute for Medical Research.
2. The Occurrence of *Cercaria clausii* Monticelli, a Marine Rattenkönig Cercaria, on the West Coast of Florida. (12 min) (Lantern) (Also by demonstration) RAYMOND M. CABLE, Purdue University, and RICHARD A. McLEAN, Academy of Natural Sciences of Philadelphia.
3. The Life Cycle of *Zoogonoides laevis* Linton, 1940. (10 min) (Lantern) HORACE W. STUNKARD, New York University.
4. The Dorso-Ventral Axis in *Taenia pisiformis*. (10 min) (Lantern) JUSTUS F. MUELLER, New York State College of Forestry.
5. *Stempellia moniezi* n. sp., a Microsporidian (Protozoa; Sporozoa) Parasite of Cestodes. (10 min) (Lantern) ARTHUR W. JONES, University of Virginia.
6. Intestinal Parasitic Infections in a State Mental Hospital, and Control with Aluminum Hydroxide and Colloidal Kaolin. (10 min) HERMAN A. SHELANSKI, W. L. PIOUS AND JESS H. FRANK, University of Pennsylvania and the Philadelphia State Hospital.
7. Anaerobiosis and Cholesterol as Growth Requirements of *Endamoeba histolytica*. (15 min) (Lantern) THOMAS L. SNYDER AND HENRY E. MELENEY, New York University.

¹ An alphabetical author index will be found at the end of this program, following which is the program (without abstracts) of a Symposium on Tropical Diseases. Extra copies of this Supplement, and portraits of parasitologists, will be on sale at the meeting.

8. Studies on Reducing Substances and Gas Formation in Cultures of *Endamoeba histolytica* and a Single Species of Symbiotic Bacteria. (10 min) (Lantern) THEODOR VON BRAND, CHARLES W. REES, LEON JACOBS AND LUCY V. REARDON, National Institute of Health.

9. Morphological Characters of the Trichomonad Flagellates of Man. (15 min) (Lantern) DAVID H. WENRICH, University of Pennsylvania.

10. *Trichomonas vaginalis* in Tissue Cultures. (15 min) (Motion picture) M. J. HOGUE, University of Pennsylvania.

11. Report on the JOURNAL OF PARASITOLOGY. (15 min) NORMAN R. STOLL, Chairman, Editorial Committee.

By Title

12. The Life Cycles of Three Dermatitis-Producing Cercariae (Trematoda: Schistosomatidae). DONALD B. McMULLEN, Michigan Stream Control Commission and University of Oklahoma, AND PAUL C. BEAVER, Michigan Stream Control Commission.

13. Methods of Investigation on the Life Cycles of Avian Schistosomes. PAUL C. BEAVER, Michigan Stream Control Commission and Georgia Department of Public Health, AND DONALD B. McMULLEN, Michigan Stream Control Commission and University of Oklahoma.

14. Studies on Cercariae of the Common Mud-flat Snail, *Cerithidea californica*. II. General Infection Data. WANDA SANBORN HUNTER, University of California at Los Angeles.

15. Taxonomic Value of the Tooth Formula of *Xironodrilus* (Branchiobdellidae). CLARENCE J. GOODNIGHT, University of Illinois.

16. Hydrogen-Ion Concentration of Amoebic Ulcers. GORDON H. BALL, University of California at Los Angeles.

17. Intravaginal Survival of Culture *Trichomonas tenax* (*buccalis*) in Man. ROBERT M. STABLER, University of Pennsylvania, AND L. G. FEO, Jefferson Medical College Hospital.

18. Inoculations of *Trichomonas foetus* (Protozoa) in Guinea Pigs. BANNER BILL MORGAN, University of Wisconsin.

TUESDAY MORNING SESSION, DECEMBER 29, 9:30 A.M.; ROOM 919, HUNTER COLLEGE, 695 PARK AVENUE.

Read

19. Exoerythrocytic Schizogony in a New Species of Saurian *Plasmodium*. (12 min) (Lantern) PAUL E. THOMPSON AND CLAY G. HUFF, University of Chicago.

20. The Effects of Thio-Bismol upon Three Species of Therapeutic Malaria. (10 min) (Lantern) MARTIN D. YOUNG, United States

Public Health Service, SOL B. McLENDON, AND ROY G. SMARR, South Carolina State Hospital.

21. The Mechanism of Quinine Action in Malaria. (15 min) (Lantern) EMANUEL WALETZKY AND HAROLD W. BROWN, University of North Carolina.

22. The Influence of Biotin upon Susceptibility to Malaria. (15 min) (Lantern) WILLIAM TRAGER, Rockefeller Institute for Medical Research.

23. The Coccidial Nature of "Avian *Toxoplasma*." (15 min) FREDERICK COULSTON, University of Chicago.

24. Maintenance of Human *Toxoplasma* in Chicken Embryos. (10 min) (Lantern) FRUMA WOLFSON, Johns Hopkins University.

25. Minimum Heat Treatment Required for the Destruction of Trichinae in Pork Scraps in Garbage. (10 min) (Lantern) WILLARD H. WRIGHT AND JOHN BOZICEVICH, National Institute of Health.

TUESDAY MORNING, DECEMBER 29, 11:00 A.M.; ROOM 919, HUNTER COLLEGE, 695 PARK AVENUE.

Presidential Address

26. The Rôle of Parasitologists in World War II. HENRY E. MELENEY, New York University College of Medicine.

TUESDAY, DECEMBER 29.

12:30 P.M. PARASITOLOGISTS' LUNCHEON, HUNTER COLLEGE.

1:30 P.M. ANNUAL BUSINESS MEETING.

TUESDAY AFTERNOON SESSION, DECEMBER 29, 3:00 P.M.; ROOM 801, HUNTER COLLEGE, 695 PARK AVENUE. (Tea will be served.)

By Demonstration

2. The Occurrence of *Cercaria clausii* Monticelli, a Marine Rattenkönig Cercaria, on the West Coast of Florida. (Also read) RAYMOND M. CABLE, Purdue University, AND RICHARD A. McLEAN, Academy of Natural Sciences of Philadelphia.

27. The Effect of Long Ultraviolet and Near Visible Radiation on the Eggs of the Nematodes, *Enterobius vermicularis* and *Ascaris lumbricoides*. MYRNA F. JONES AND ALEXANDER HOLLAENDER, National Institute of Health.

28. *Phyllodistomum etheostomae* n. sp. (Trematoda: Gorgoderidae) from Percid Fishes. JACOB H. FISCHTHAL, University of Michigan.

36. Some Preliminary Experiments on a Skin Test for Immunity to the Stomach Worm, *Haemonchus contortus*, in the Calf. (Also read) ROY L. MAYHEW, Louisiana State University.

65. African Sleeping Sickness. (30 min) (Lantern; motion picture) EUGENE R. KELLERSBERGER, American Mission to Lepers, New York (4:00 P.M., Room 919, Hunter College) (By invitation).

WEDNESDAY MORNING SESSION, DECEMBER 30, 9:30 A.M.; ROOM 919, HUNTER COLLEGE, 695 PARK AVENUE.

Read

29. Increased Mortality in Rats with Concomitant Dual Infections. (10 min) GRAEME A. CANNING AND J. M. FISHER, University of Tennessee.
30. The Effect of Dry-Heating the Ration on Oöcyst Production in *Eimeria nieschulzi* Infections. (12 min) (Lantern) ELERY R. BECKER, Iowa State College.
31. Effect of X-Irradiation upon the Resistance of Rats to *Trypanosoma lewisi*. (10 min) (Lantern) DOROTHY N. NAIMAN, Hunter College, and Columbia University.
32. Number of Larvae and Time Required to Produce Active Immunity in Rats against *Trichinella spiralis*. (10 min) (Lantern) JACOB H. FISCHTHAL, State University of Iowa.
33. *Trichinella* Skin Tests in Apparently Normal Individuals. (10 min) (Lantern) HARRY M. ROSE AND JAMES T. CULBERTSON, Columbia University.
34. Immunological Studies on a Polysaccharide and Protein Fraction Isolated from *Trichinella spiralis*. (15 min) (Lantern) LEO R. MELCHER, University of Chicago. (Introduced by CLAY G. HUFF.)
35. Active Immunization of Sheep against Large Single Test Infections of *Haemonchus contortus*. (15 min) (Lantern) NORMAN R. STOLL, Rockefeller Institute for Medical Research.
36. Some Preliminary Experiments on a Skin Test for Immunity to the Stomach Worm, *Haemonchus contortus*, in the Calf. (15 min) (Lantern) (Also by demonstration) ROY L. MAYHEW, Louisiana State University.
37. Acquired Resistance to the Gapeworm, *Syngamus trachea*, in the Turkey and Ring-Necked Pheasant. (12 min) (Lantern) LOUIS OLIVIER, United States Bureau of Animal Industry.
38. Skin Tests in Schistosomiasis *Mansoni* Patients with Antigen from *Pneumoneces medioplexus*. (5 min) (Lantern) JAMES T. CULBERTSON AND HARRY M. ROSE, Columbia University.
39. Resistance to *Ascaris lumbricoides* L. in Guinea Pigs and the Eosinophilia Associated with Infection. (15 min) (Lantern) A. MURRAY FALLIS, Ontario Research Foundation.

By Title

40. The Relation of Splenectomy and the Resistance of Old Mice to the Mouse Cestode, *Hymenolepis nana* var. *fraterna*. JOHN E. LARSH, JR., Johns Hopkins University.

41. The Effect of *Ascaris suum* Extract Injections upon Chickens Infected with *Ascaridia lineata*. L. L. EISENBRANDT, University of Kansas City.

42. Effects of the Nematode, *Trichostrongylus colubriformis*, on the Nutrition of Lambs. JOHN S. ANDREWS, W. R. KAUFFMAN, AND R. E. DAVIS, United States Bureau of Animal Industry.

43. Symptoms and Immunity Following Graduated Doses of *Eimeria tenella*. HARRY A. JANKIEWICZ, University of Southern California.

44. Studies on Susceptibility of Chickens to Cecal Coccidiosis. C. A. HERRICK AND S. A. EDGAR, University of Wisconsin.

WEDNESDAY AFTERNOON SESSION, DECEMBER 30, 2:00 P.M.; ROOM 919,
HUNTER COLLEGE, 695 PARK AVENUE.

Read

45. The Magnitude of the Hookworm Problem in a Typical County of Southern Georgia. (12 min) (Lantern) J. ALLEN SCOTT AND JUSTIN ANDREWS, Georgia Department of Public Health.

46. Use of Calcium Chloride to Isolate Helminth Ova from Soil. (10 min) ROBERT T. STEVENSON, University of Wisconsin. (Introduced by C. A. HERRICK.)

47. Phenothiazine in the Treatment of Enterobiasis. (10 min) ELLA KUITUNEN, University of Toronto. (Introduced by A. MURRAY FALLIS.)

48. The Efficacy of Phenothiazine and Nicotine-Bentonite for the Removal of *Heterakis gallinae* and *Ascaridia galli* from Chickens. (15 min) (Lantern) JAMES E. GUTHRIE AND PAUL D. HARWOOD, Dr. Hess and Clark Laboratories.

49. Observation on the Life History of a Rabbit Cuterebrid, the Larvae of Which May Penetrate the Human Skin. (12 min) (Lantern) RAYMOND H. BEAMER, University of Kansas, AND LAWRENCE R. PENNER, University of Connecticut.

50. On the Structure of So-Called "Stigmata" of Larval Ticks. (5 min) (Lantern) HAROLD ELISHEWITZ, University of Minnesota.

51. The Taxonomy of the Pathogenic Rickettsiae. (10 min) (Lantern) CORNELIUS B. PHILIP, Army Medical School.

52. Age as a Factor in the Resistance of Splenectomized Rats to Infection with *Bartonella muris*. (10 min) (Lantern) WALTER R. KESSLER, Columbia University, and College of the City of New York.

53. The Pathogenicity of Three Strains of *Trichinella spiralis* as Indicated by Lethal Dose and Survival Time. (15 min) (Lantern) IRVING RAPPAPORT, Cornell University.

54. Tests for a Hemolytic Substance in *Chabertia ovina*. (15 min) (Lantern) W. L. THRELKELD, Virginia Agricultural Experiment Station.

55. Factors Influencing the Oxygen Consumption of a Larval *Eustrongylides*. (10 min) (Lantern) THEODOR VON BRAND, Catholic University of America.

By Title

56. Survival on Soil of Oöcysts of Two Species of Swine Coccidia, *Eimeria deblickei* and *E. scabra*. JOHN LAWRENCE AVERY, United States Bureau of Animal Industry.

57. The Effect of Moderately Low Temperatures on the Sporulation of Oöcysts of Two Species of Swine Coccidia. JOHN LAWRENCE AVERY, United States Bureau of Animal Industry.

58. The Segmental Anatomy of *Mesocetoides variabilis* Mueller, 1928, from *Didelphis virginiana* Kerr. ELON E. BYRD, University of Georgia, AND JAMES W. WARD, Mississippi State College.

59. Notes on the Genital System of the Bird Fluke, *Apharyngostrigea cornu* (Zeder). ELON E. BYRD, University of Georgia, AND JAMES W. WARD, Mississippi State College.

60. The Parasites of the Caecilidae (Amphibia : Apoda). A. C. WALTON, Knox College.

61. The Parasites of the Cryptobranchoidea (Amphibia : Caudata). A. C. WALTON, Knox College.

62. The Parasites of the Ambystomoidea (Amphibia : Caudata). A. C. WALTON, Knox College.

63. The Possibility of Chemical Control of the Snail Intermediate Hosts of *Schistosoma mansoni* in Venezuela. II. GEORGE W. LUTTERMOSER, Instituto Nacional de Higiene, Caracas, Venezuela.

64. A Peculiar Larval Development of *Rhabdochona* spp. (Nematoda : Spiruroidea). PAUL V. GUSTAFSON, Whitworth College.

By Invitation

65. African Sleeping Sickness. (30 min) (Lantern; motion picture) EUGENE R. KELLERSBERGER, American Mission to Lepers, New York (Tuesday, December 29, 4:00 P.M.; Room 919, Hunter College.)

AUTHOR INDEX

7

AUTHOR INDEX

Showing program number, which is also the abstract number of each paper.

<i>Author</i>	<i>Program and Abstract Number</i>	<i>Author</i>	<i>Program and Abstract Number</i>
Andrews, John S.	42	Kuitunen, Ella	47
Andrews, Justin	45	Larsh, John E., Jr.	40
Avery, John Lawrence	56, 57	Luttermoser, George W.	63
Ball, Gordon H.	16	Mayhew, Roy L.	36
Beamer, Raymond H.	49	McLean, Richard A.	2
Beaver, Paul C.	12, 13	McLendon, Sol B.	20
Becker, Elery R.	30	McMullen, Donald B.	12, 13
Bozicevich, John	25	Melcher, Leo R.	34
Brown, Harold W.	21	Meleney, Henry E.	7, 26
Byrd, Elon E.	58, 59	Morgan, Banner B.	18
Cable, Raymond M.	2	Mueller, Justus F.	4
Canning, Graeme A.	29	Naiman, Dorothy N.	31
Coulston, Frederick	23	Olivier, Louis	37
Culbertson, James T.	33, 38	Penner, Lawrence R.	49
Davis, R. E.	42	Philip, Cornelius B.	51
Edgar, S. A.	44	Pious, W. L.	6
Eisenbrandt, L. L.	41	Rappaport, Irving	53
Elishewitz, Harold	50	Reardon, Lucy V.	8
Fallis, A. Murray	39	Rees, Charles W.	8
Feo, L. G.	17	Rose, Harry M.	33, 38
Ferguson, M. S.	1	Scott, J. Allen	45
Fischthal, Jacob H.	28, 32	Shelanski, Herman A.	6
Fisher, J. M.	29	Smarr, Roy G.	20
Frank, Jess H.	6	Snyder, Thomas L.	7
Goodnight, Clarence J.	15	Stabler, Robert M.	17
Gustafson, Paul V.	64	Stevenson, Robert T.	46
Guthrie, James E.	48	Stoll, Norman R.	11, 35
Harwood, Paul D.	48	Stunkard, Horace W.	3
Herrick, C. A.	44	Thompson, Paul E.	19
Hogue, M. J.	10	Threlkeld, W. L.	54
Hollaender, Alexander	27	Trager, William	22
Huff, Clay G.	19	von Brand, Theodor	8, 55
Hunter, Wanda S.	14	Waletzky, E.	21
Jacobs, Leon	8	Walton, A. C.	60, 61, 62
Jankiewicz, Harry A.	43	Ward, James W.	58, 59
Jones, Arthur W.	5	Wenrich, David H.	9
Jones, Myrna F.	27	Wolfson, Fruma	24
Kauffman, W. R.	42	Wright, Willard H.	25
Kellersberger, Eugene R.	65	Young, Martin D.	20
Kessler, Walter R.	52		

SYMPOSIUM ON TROPICAL DISEASES

Program of Section N (Medical Sciences) of the A. A. A. S., Organized in Cooperation with the American Society of Parasitologists, the National Malaria Committee, the American Society of Tropical Medicine, and the New York Society of Tropical Medicine

NEW YORK, NEW YORK

TUESDAY, DECEMBER 29, 1942

PROGRAM²

MORNING SESSION, DECEMBER 29, 9: 30 A.M.; HOTEL COMMODORE.

1. Tropical Diseases and Military Medicine. COLONEL JAMES S. SIMMONS, Office of the Surgeon General, War Department, Washington, D.C.
2. The Implications of Tropical Medicine to the Civilian Population. RICHARD P. STRONG, Army Medical School, Washington, D. C.
3. The Teaching of Tropical Medicine in the United States. LIEUTENANT-COLONEL THOMAS T. MACKIE, Army Medical School, Washington, D. C.
4. The Role of Tropical Medicine in International Affairs. ALAN GREGG, Rockefeller Foundation, New York, N. Y.
5. THE THEOBALD SMITH AWARD. Presentation by PRESIDENT COMPTON of the A. A. A. S. Address by Recipient.

AFTERNOON SESSION, DECEMBER 29, 2: 00 P.M.; HOTEL COMMODORE.

6. Dysenteries and Diarrheas—Their Importance in the Military Service. COLONEL G. R. CALLANDER, Army Medical Center, Washington, D. C.
7. Relapsing Fever. MALCOLM H. SOULE, University of Michigan Hygienic Laboratory, Ann Arbor, Michigan.
8. Malaria Control. LOWELL T. COGGESHALL, University of Michigan Institute of Public Health, Ann Arbor, Michigan.
9. Yellow Fever. WILBUR A. SAWYER, Rockefeller Foundation, International Health Division, New York, N. Y.

ADDRESS OF VICE PRESIDENT OF SECTION N

10. The Man Who Lived for Tomorrow. WADE W. OLIVER, The Long Island College of Medicine, Brooklyn, New York.

EVENING SESSION, DECEMBER 29, 8: 30 P.M.; HOTEL COMMODORE.

THEOBALD SMITH LECTURE OF THE NEW YORK SOCIETY
OF TROPICAL MEDICINE

11. Typhus Fever. M. RUIZ CASTAÑEDA, General Hospital, Mexico City, Mexico.

² No abstracts.

ABSTRACTS

AMERICAN SOCIETY OF PARASITOLOGISTS' PROGRAM

1. *Development of the Metacercariae of Diplostomum flexicaudum in the Lenses of Frogs, Turtles, Birds, and Mammals.* M. S. FERGUSON, Rockefeller Institute for Medical Research.

Frog tadpoles, frogs, turtles, chicks, ducklings, and laboratory white mice, rats, guinea pigs, and rabbits were exposed to the cercariae of the strigeid trematode *Diplostomum flexicaudum*. The cercariae were able to penetrate the eye and grow in the lenses of every species. The metacercariae which developed were normal in appearance and behavior and were similar to those found in the eyes of fishes, the normal intermediate hosts. There was usually a considerable variation in size among metacercariae from each particular lens as well as among metacercariae from the eyes of different species of animals. Similar size variations were found in metacercariae from the eyes of fishes. Metacercariae from the lenses of a frog and a guinea pig were infective for baby chicks, and normal adult trematodes with eggs were recovered from them after one week. Blindness was caused in many of the infected eyes. Initial damage resulted in an opacity of the cortex and nucleus of the lens. In some cases the cortical substance later became partially liquified. Diseased lenses usually were evident earlier, and due to fewer larval trematodes, in the warm-blooded animals than in the cold-blooded ones. Certain experiments and an apparent complete lack of records in medical literature suggest that the possibility of these metacercariae causing disease in man is rather remote. However, it remains to be proved that such a danger does not exist.

2. *The Occurrence of Cercaria clausii Monticelli, a Marine Rattenkönig Cercaria, on the West Coast of Florida.* RAYMOND M. CABLE, Purdue University, and RICHARD A. McLEAN, Academy of Natural Sciences of Philadelphia.

A marine rattenkönig cercaria has been found to parasitize a species of *Lamellaria* collected near Captiva Island, Florida. This larva agrees in all essential respects with *Cercaria clausii*, the only known marine larval trematode of this type and hitherto reported only from the Adriatic region. The cercariae develop in simple daughter rediae in the digestive gland of the snail. Very small mother rediae in the mantle produce daughter rediae one at a time. There is no indication of rosette formation within the daughter redia; the cercariae develop singly just as do other species. Since there are many fundamental differences between *C. clausii* and *C. gorgonocephala*, the only known species of freshwater rattenkönig cercaria, it appears that rosette formation may not indicate close affinities but instead a larval adaptation that may arise independently in distantly related groups. The discovery of a pharynx in *C. clausii* removes the possibility that this species is the larva of *Phyllodistomum acceptum*, a marine gorgoderid, as claimed by Odhner. *C. clausii* possesses several characters in common with the trichocercous cercariae, viz., eyespots, a well-developed ventral sucker, a sac-shaped, thick-walled excretory bladder, and hair-like processes on the tail. For this reason, the adult stage probably should be sought among fish parasites belonging to the family Lepocreadiidae or the closely related family Gyliarchaeidae.

3. *The Life Cycle of Zoogonoides laevis Linton, 1940.* HORACE W. STUNKARD, New York University.

Sexually mature specimens of *Zoogonoides laevis* occur regularly and abundantly in the intestine of *Tautoga onitis*. Eggs in the uterus have membranous coverings but no shells, and when passed they contain active miracidia. The larvae emerge almost immediately in sea water and *Mitrella lunata* serves as the first intermediate host. There are at least two generations of sporocysts, the last of which produces cercariae. The cercariae are terete, tailless, with spined cuticula, simple stylet, anterior setiferous papillae, acetabulum slightly larger than oral sucker,

numerous penetration and cystogenous glands. The flame cell pattern is $[(2+2) + (2+2)]$. Snails were infected in the laboratory and at the end of seven weeks, although they contained young cercariae, they had not yet produced mature cercariae which could be used to complete the cycle. But cercariae from naturally infected snails encysted readily in *Nereis virens*. *Nereis* exposed to cercariae for two weeks were fed to tautogs which had been isolated for seven weeks. The fishes were dissected two days later and many young *Z. laevis* were recovered. These small specimens were clearly distinguishable from larger specimens of natural infection which were present in the intestine of the fish when they were caught.

4. *The Dorso-Ventral Axis in Taenia pisiformis*. JUSTUS F. MUELLER, New York State College of Forestry.

It is generally stated in standard reference works that one entire surface of the cestode strobila may be considered ventral, the other dorsal, and that the ovary lies nearest the ventral surface. In the taenias two pairs of longitudinal excretory vessels occur. The two larger excretory vessels, connected near the posterior margin of each proglottid, are usually designated as the ventral excretory vessels. The smaller vessels, without transverse connections, are usually designated as dorsal. In a restudy of the anatomy of *Taenia pisiformis* from the dog it was discovered that these relationships are inconstant. Using the ovary to determine orientation, in few proglottids do the large excretory trunks lie ventrally. The most frequent condition is with the large canal ventral to the small canal on one margin, dorsal to it on the other, with the transverse connections passing diagonally. In other cases both small vessels lie ventral in position, the large canals dorsal. In certain instances the ovary reverses its position with reference to the surfaces of the strobila in adjacent proglottids, thus reversing the dorso-ventral axis. It is clear, therefore, that neither the relationship of the excretory canals to each other, nor the orientation of the dorso-ventral axis is constant throughout the length of the strobila. Nor can any character be found that bears any constant relationship to one surface or the other. It is impossible, therefore, in *T. pisiformis* at least, to differentiate between the two surfaces of the strobila, or to designate either surface as dorsal or ventral.

5. *Stempellia moniezi n. sp., a Microsporidian (Protozoa: Sporozoa) Parasite of Cestodes*. ARTHUR W. JONES, University of Virginia.

In two cestodes, *Hymenolepis anthocephalus* Van Gundy, 1936, and *Diorchis reynoldsi* (description in press), taken in 1941 from the intestine of the shrew, *Blarina brevicauda* Say, 1823, a protozoan parasite was found. The previous reports of protozoa from the cestodes were made prior to 1900, and are not sufficiently detailed to classify properly any of the reported forms. In recognition, however, that Moniez (1887) gave the first name and description (although inadequate) to a protozoan parasite of cestodes, the new species is named in his honor.

The protozoon infects the parenchyma of its cestode host, lying chiefly near the excretory ducts. Its spores, of general size 4.0–5.0 micra by 1.5–2.0 micra, are thin-walled, subcylindrical. They develop singly or in groups of two, four, and eight, from a single sporoblast; a size gradation is evident, the spores of a group of eight being the smallest, as is characteristic of the genus *Stempellia* Leger et Hesse, 1922, (of the Family Nosematidae Labbé, 1899, Order Microsporidia Balbiana, 1882), according to Kudo (1924). It is suggested that the infection of cestodes is accidental, and that the normal host of this protozoon may be, as is usual in the Nosematidae, a larval insect.

6. *Intestinal Parasitic Infections in a State Mental Hospital, and Control with Aluminum Hydroxide and Colloidal Kaolin*. HERMAN A. SHELANSKI, W. L. PIOUS AND JESS H. FRANK, University of Pennsylvania and the Philadelphia State Hospital.

In a routine study of the bloods of several hundred patients at the Philadelphia State Hospital it was found that an unusually high number of patients showed a

markedly high eosinophilia, a sign indicative of infection of a parasitic nature. It was then decided to make fecal examinations of the patients showing this high eosinophilia in order to determine whether or not there was present any gastro-intestinal infection.

After fecal examination was made it was found that all of the patients who showed a markedly high eosinophilia were infected with one or more species of parasites. The following types of infection were found: *Trichuris trichiura*, *Oxyuris vermicularis*, *Ascaris lumbricoides*, *Strongyloides stercoralis*, *Hymenolepis nana*, *Endamoeba histolytica*, *Endamoeba coli*, *Endolimax nana*, *Iodamoeba bütschlii*, *Dientamoeba fragilis*, *Retortamonas hominis*, *Chilomastix mesnili*, *Giardia lamblia*, and *Trichomonas intestinalis*.

A suspension of aluminum hydroxide and colloidal kaolin was used in these cases to rid them of these infections. The patients were given three one-ounce doses daily. This medication was continued for a period of three to four weeks with examinations being made at weekly intervals during this period. At the end of this time fecal examinations made every week for a period of four to eight weeks showed that most of these cases were free of parasites. The only organism which persisted was *Trichuris trichiura*, since on repeated examination ova of this parasite were found in the feces.

7. *Anaerobiosis and Cholesterol as Growth Requirements of Endamoeba histolytica*. THOMAS L. SNYDER AND HENRY E. MELENEY, New York University.

A study of the growth requirements of *Endamoeba histolytica* is being conducted with cultures of the motile amoebae containing bacteria. A fluid medium consisting of a saline infusion of coagulated whole egg produced satisfactory growth under aerobic conditions and was used as a basis for studying individual requirements. Since excystation in sterile media occurred only under anaerobic conditions it seemed advisable to study the motile amoebae under similar conditions. Successive anaerobic transplants to sterile egg infusion medium were unsuccessful, apparently because of a decrease in the bacterial flora. They were successful, however, when the medium was seeded with the original bacterial flora and incubated aerobically for 24 hours prior to transplanting the amoebae for anaerobic cultivation. Subsequently it was found that a reducing agent (cysteine) could be substituted for the additional bacteria thus furnished, and that peptone in Ringer's solution plus cholesterol (1 part per million) could be substituted for the fluid portion of the culture. Rice starch was necessary in all anaerobic cultures. Exposure of anaerobic cultures to air for approximately one hour caused death of the amoebae, thus confirming their strict anaerobic requirements. The anaerobic amoeba cultures in this peptone-Ringer-cholesterol-cysteine-starch medium contain a diminished bacterial flora which furnishes some undiscovered substance or condition necessary for the cultivation of *E. histolytica*. This flora has been further simplified by obtaining a successful anaerobic amoeba culture from bacteria-free cysts with a single species of anaerobic bacillus recovered from the original amoeba culture.

8. *Studies on Reducing Substances and Gas Formation in Cultures of Endamoeba histolytica and a Single Species of Symbiotic Bacteria*. THEODOR VON BRAND, CHARLES W. REES, LEON JACOBS AND LUCY V. REARDON, National Institute of Health.

Using the micro-method of Hagedorn and Jensen, it was found that the raw egg emulsion of the L.E.S. medium contains 278 to 312 mgm per cent of reducing substances. In tubes, diffusion of these substances from the egg slant to the saline overlay takes place over a period of a week, with a final concentration in the liquid of about 50 mgm per cent; diffusion in flasks is much more rapid. In media to which glucose in amounts up to 300 mgm per cent was added to the overlay, the diffusion was less rapid and of less degree. In cultures of *Endamoeba histolytica* grown with a single bacterial symbiont and in cultures of the symbiont alone, to neither of which glucose was added, the reducing substances were almost completely

utilized, leaving only 7 to 19 per cent after 24 hours. In media with glucose in the overlay, most of the sugar was utilized in 24 to 48 hours. No differences could be detected in utilization of reducing substances in cultures of the amoeba and the bacterium or in cultures of the bacterium alone. In the presence of glucose, gas was produced in like amount and composition in cultures of the bacterium alone and in cultures of the amoeba plus the bacterium. The predominant constituent was hydrogen (70.1 to 74.7 per cent), indicating an anaerobic metabolism. The other gases were CO_2 (11.2 to 18.9 per cent), O_2 (0.3 to 1.3 per cent), N_2 (5.9 to 14.1 per cent), and CH_4 (0.2 to 1.3 per cent).

9. *Morphological Characters of the Trichomonad Flagellates of Man.* DAVID H. WENRICH, University of Pennsylvania.

Trichomonas vaginalis Donné, largest of the trichomonads of man, has 4 anterior flagella; costa and undulating membrane are one-third to two-thirds of the body length; marginal filament is double; there is no trailing flagellum. Parabasal apparatus consists of a long parabasal fibril and a shorter, sausage-shaped parabasal body; a shorter, additional parabasal body is often seen in some populations. The relatively slender axostyle may split into 2 or more splinters in larger individuals. Nucleus is usually elongated, with a small endosome. Cytoplasm of stained specimens generally contains many chromatic granules.

T. tenax (O. F. Müller) from the mouth is smaller but similar to *T. vaginalis* in organization. It has 4 anterior flagella, less-than-body-length costa and undulating membrane, double marginal filament, parabasal fibril and parabasal body, and slender axostyle. Nucleus is usually rounded. Fewer chromatic granules occur in the cytoplasm.

T. hominis (Davaine) from the intestine is intermediate in size; has 4 or 5 anterior flagella; costa and undulating membrane are body-length; marginal filament is double; trailing flagellum present; and at least one row of paracostal granules; parabasal apparatus not yet identified; axostyle relatively coarser than in two above species. Similar and apparently identical forms occur in monkeys, dogs, cats and rats.

T. fecalis Cleveland has 3 long anterior flagella; body-length costa and undulating membrane; double marginal filament; long trailing flagellum; two or more rows of paracostal granules; axostyle relatively thick, dense and long. It is similar, if not identical to *T. batrachorum* (?) from frogs and toads.

10. *Trichomonas vaginalis in Tissue Cultures.* M. J. HOGUE, University of Pennsylvania.

Dr. Trussell's pure culture of *Trichomonas vaginalis* was introduced into tissue cultures of chick embryo intestine. This movie shows the trichomonads swarming along the cut edges of the embryonic tissues and entering the lumen of pieces of the intestine. Many individuals clump together to form rosettes. The trichomonads attach themselves with their axostyles to epithelial cells and whirl about on them. They wander about in a plexus of sympathetic nerves, resting on the nerves with their axostyles. In feeding over the tissue culture cells they often send out a proboscis-like structure containing the flagella and undulating membrane. In the hanging drop they drag and push about the plasma granules. They do not mechanically injure the tissue culture cells.

11. *Report on the JOURNAL OF PARASITOLOGY.* NORMAN R. STOLL, Rockefeller Institute for Medical Research.

A five-year perspective, and the impact of the war viewed from the office of the Chairman, Editorial Committee.

12. *The Life Cycles of Three Dermatitis-Producing Cercariae (Trematoda: Schistosomatidae).* DONALD B. McMULLEN, Michigan Stream Control Commis-

sion and University of Oklahoma, AND PAUL C. BEAVER, Michigan Stream Control Commission.

Adult schistosomes and ova were obtained in experimental animals exposed to *Cercaria stagnicola* Talbot, *Cercaria elvae* Miller and *Cercaria physellae* Talbot. The worms reached maturity in birds exposed for 8-25 days to the cercariae in drinking water. Experiments indicated, however, that the cercariae can also penetrate the external surface of birds and establish infections. In 25-48 days after exposure to *C. stagnicola* trematodes belonging to the genus *Trichobilharzia* Skrjabin and Zakharow were recovered from the peripheral veins of the intestine of two canaries. Embryonated ova were numerous in the gut. A Caspian tern, chickens, domestic ducklings, mallards, and gulls exposed to the cercariae were negative. Great numbers of another *Trichobilharzia* sp. were found in the venous plexus of the intestinal mucosa and extending into the villi of two mallards, five domestic ducklings, and two canaries exposed to *C. elvae*. Fully embryonated ova appeared about two weeks after exposure. The ducks were much more susceptible than the canaries. In experiments with a Caspian tern, pigeons, and chickens, the worms failed to mature. Trematodes belonging to the genus *Pseudobilharzia* Ejsmont were recovered in large numbers from the peripheral veins of the intestine and the venous plexus of the submucosa in two pigeons, two mallards, and three canaries 2-4 weeks after exposure to *C. physellae*. In one heavily infected canary large numbers of ova and 300-400 worms were found in the intestine, liver and lungs. A Caspian tern, chickens, domestic ducklings, and gulls exposed to this cercaria were negative.

13. *Methods of Investigation on the Life Cycles of Avian Schistosomes.* PAUL C. BEAVER, Michigan Stream Control Commission and Georgia Department of Public Health, AND DONALD B. McMULLEN, Michigan Stream Control Commission and University of Oklahoma.

The experimental birds were exposed by providing daily fresh suspensions of cercariae, *Cercaria stagnicola* Talbot, *C. physellae* Talbot and *C. elvae* Miller, in the drinking water. Infections were obtained also by immersion in cercarial suspensions, but in well-feathered hosts the breast and leg feathers were removed. To determine the presence of mature infections, to discover light infections at autopsy, and to concentrate miracidia for other experiments, fecal samples were emulsified in small quantities of water in one-liter, vertical side arm distillation flasks. The flasks were then filled to the side arm with tap water, allowed to settle, then completely filled by rapidly pouring into the side arm. A paper bag with a hole just large enough and at the proper level to accommodate the side arm was placed over each flask, leaving the arm exposed to light while the remainder was in darkness. The miracidia, being negatively geotropic and positively phototropic, were readily observed as they collected in the clear water of the side arm. In general the search for the adult worms, fixation, and staining were done in the manner described by Brackett (1942). It was found that the worms were easier to find and remained active for several hours if the vessels were injected with one per cent sodium citrate in physiological salt solution immediately after killing the host. This was done by using a gravity bottle and injecting from the heart until the blood in the tributaries of the mesenteric veins was highly diluted.

14. *Studies on Cercariae of the Common Mud-flat Snail, Cerithidea californica.* II. *General Infection Data.* WANDA SANBORN HUNTER, University of California at Los Angeles.

The area chosen for detailed study was Anaheim Slough, located on U. S. Highway 101, approximately one mile south of Seal Beach, California. During a 2-year period, 3934 *Cerithidea californica* were brought into the laboratory and detailed study of their cercarial fauna made. Percentages of snails shedding apparently mature cercariae, those harboring parthenitae and maturing cercariae, and the percentages of total infection were determined. Percentages of total infection were

computed by the formula $P = \frac{pN + S}{T}$. P = total percentage; p = percentage of infection

obtained by crushing; N = number of snails negative for shedding when isolated; S = number of snails shedding; and T = N + S. For the 2-year period, high peaks in total infection were found in April and in November. The peaks are explained by environmental factors such as temperature (which plays a minor rôle in this region), the daily tides, rainfall, salinity of water, and bird migrations.

The percentage of infection in the entire catch for all collections was estimated as 32.6. However, there was too great a variation from month to month to assume a constant percentage of infection. Analysis of the type of infection revealed that 33 per cent were pleurolophocercous cercariae, 18 per cent echinostomes, 18 per cent xiphidiocercariae, and but 6.5 per cent were furcocercous forms. Correlations between the amount of infection found in the aforementioned groups and environmental factors were made. Five and one-half per cent of the infected snails yielded multiple infections. Comparisons were made between Anaheim Slough collections and those from 3 other areas in Southern California.

15. *Taxonomic Value of the Tooth Formula of Xironodrillus (Branchiobdellidae)*. CLARENCE J. GOODNIGHT, University of Illinois.

Additional material studied has enabled the writer to evaluate the taxonomic importance of the teeth formulae of the genus *Xironodrillus*. Ellis (1919) while studying numerous specimens of *X. pulcherrimus* Moore found many individuals which had a 4-4 to a 5-5 tooth formula rather than the 3-3 of the type. Ellis considered these as variations in a single species. Studies by the writer (1940) persuaded him that actually two subspecies were represented: *pulcherrimus* and *dentatus*. Further material has shown these differences to be constant; so that actually two species, rather than one variable one had to be considered. A new eastern species encountered exhibited the same tooth formula as *X. pulcherrimus*; however the relative size of the teeth differed. Thus the writer has come to the conclusion that even in a genus with such variable teeth formulae as *Xironodrillus*, species differences may be recognized by the formula and the relative sizes of the teeth.

16. *Hydrogen-ion Concentration of Amoebic Ulcers*. GORDON H. BALL, University of California at Los Angeles.

By the use of the capillary glass electrode, it is possible to determine the hydrogen-ion concentration of amoebic ulcers in live kittens and to compare this reading with that of the normal gut wall in the same animal or with that of wounds produced mechanically. In any one animal, an amoebic ulcer or a mechanical wound in the cecum or the colon shows usually a lower pH than does the uninjured wall in the same region of the gut. However, these readings are not beyond the range of the pH changes in the lumen which are found at times in normal animals. It is doubtful if pH differences play a significant role in maintaining *Endamoeba histolytica* in an ulcer instead of in the gut lumen. The pH differences between normal and infected tissue disappear rapidly after the death of the animal.

17. *Intravaginal Survival of Culture Trichomonas tenax (buccalis) in man*. ROBERT M. STABLER, University of Pennsylvania, AND L. G. FEO, Jefferson Medical College Hospital.

Fifty human volunteers (25 positive and 25 negative for *Trichomonas vaginalis*) were each given 3 intravaginal implantations of *T. tenax* from culture. Survivals of 48 hours were noted in 2 *T. vaginalis*-positive patients, and 8 survivals of at least this length were noted in those free of *T. vaginalis*. In this latter group 5 were of 48 hours, and one each of 72, 168 and 432 hours, respectively. No permanent vaginal infection with the oral form resulted. Four showed at least a 48-hour *T. tenax* survival after the first implantation, 2 after the second, and 2 not until after the third. In the *T. vaginalis*-free patients, *T. tenax* was present in sufficient numbers to be detected on the wet smear in 3 of the 6 showing survival, while the other 3

were proven positive by culture alone. Three patients (1 with and 2 without *T. vaginalis*) were given intravaginal inoculations directly from the mouth of a person positive for *T. tenax*. No survival was noted at 48 hours. It is concluded that the failure of the oral trichomonad to permanently establish itself in the human vagina after 150 implantations completely supports the morphological studies which indicate that the mouth and vaginal forms are two separate species.

18. *Inoculations of Trichomonas foetus (Protozoa) in Guinea Pigs.* BANNER BILL MORGAN, University of Wisconsin.

Sixty-two guinea pigs, divided into 3 groups, were inoculated intraperitoneally with: (1) washed *Trichomonas foetus* suspended in saline with a concentration of 10 million living organisms per cc; (2) liquid portion of 72-hour *T. foetus* cultures composed of buffered saline-citrate solution with 5 per cent bovine serum in which the concentration was approximately 3 million living organisms per cc; and (3) sterile trichomonad pyometra material from an infected cow with a count of 1.5 million living organisms per cc. Only 3 animals became infected. Twenty guinea pigs were refractory to subcutaneous injections of *T. foetus* in pure culture while 12 guinea pigs were positive to subcutaneous injections of sterile trichomonad pyometra material from an infected cow. The abscesses which were produced remained positive for *T. foetus* up to 42 days. Of thirty guinea pigs inoculated vaginally with sterile pyometra material, only 2 animals were positive for 5 days.

19. *Exoerythrocytic Schizogony in a New Species of Saurian Plasmodium.* PAUL E. THOMPSON AND CLAY G. HUFF, University of Chicago.

A new species of *Plasmodium* recovered from Mexican lizards (*Sceloporus ferrariperesi*) and maintained in Texas collared lizards (*Crotaphytus collaris*), in *Sceloporus undulatus*, and in horned toads (*Phrynosoma cornutum*) has the characteristic of *P. elongatum* (of birds) of inhabiting blood cell precursors, including reticular cells, and also is capable of infecting endothelial cells of the brain. It has, therefore, both *elongatum* and *gallinaceum* types of exoerythrocytic schizogonic stages. This suggests that the various species of malarial parasites may have evolved from such a primitive stock and have differentiated into species with different host cell preferences.

20. *The Effects of Thio-Bismol upon Three Species of Therapeutic Malaria.* MARTIN D. YOUNG, United States Public Health Service, SOL B. McLENDON AND ROY G. SMARR, South Carolina State Hospital.

Used in induced malaria, 0.1 or 0.2 grams of thio-bismol (sodium bismuth thiolglycollate) eliminated the paroxysms caused by broods of *Plasmodium vivax* parasites which were about one-half grown. The range of effectiveness was from 16 to 28 hours after the paroxysm. Parasites of other ages were not consistently affected. Thus it is possible to change the periodicity of paroxysms from quotidian to tertian, which is of considerable value to the neurosyphilitic patient undergoing malaria therapy. Thio-bismol did not exhibit this selective effect upon induced infections of *P. malariae* and *P. falciparum*.

21. *The Mechanism of Quinine Action in Malaria.* EMANUEL WALETZKY AND HAROLD W. BROWN, University of North Carolina.

Various quantitative aspects of the relations between quinine, the host, and the parasite have been studied for their bearing on the problem of the direct versus the indirect action of quinine. The duration of the anti-plasmodial environment following single massive intravenous doses of quinine has been determined. The degree of inhibition of asexual reproduction in *Plasmodium lophurae* introduced into ducks at different intervals following quinine administration served as the criterion of anti-plasmodial activity. The anti-plasmodial activity has been correlated with the blood and tissue levels of quinine present at similar intervals following single intravenous

doses of quinine in the duck. The minimal length of time necessary for in vivo action of quinine has also been studied. In this case, the administration of quinine was followed by the inoculation of the parasites, which were then withdrawn and introduced into clean birds. The in vitro effect of different quinine concentrations, acting for similar lengths of time, has been studied and compared with the in vivo results.

22. *The Influence of Biotin upon Susceptibility to Malaria.* WILLIAM TRAGER, Rockefeller Institute for Medical Research.

Young ducks and chickens were rendered biotin-deficient by feeding them diets containing 25 per cent dried egg white. Control animals were kept on similar diets which contained casein in place of the egg white. In some experiments animals were fed the high egg white diet plus enough biotin concentrate to provide an excess of biotin. After intravenous inoculation with blood infected with the avian malaria parasite, *Plasmodium lophurae*, the biotin-deficient animals regularly developed more severe infections than the controls. For example, in one experiment in which the chickens were 22 days old when they were inoculated, the peak parasite numbers for 12 chickens kept on an egg-white diet and showing typical signs of biotin deficiency were (per 10,000 red cells): 5940, 2420, 2100, 1440, 4000, 1660, 1080, 3280, 2940, 2660, 3680 and 5960, while the peak parasite numbers for the 6 control chickens were: 880, 1180, 800, 1440, 4000 and 1120. While 9 of the 12 chickens in the egg-white group had peaks of over 2000, this was true of only 1 of the 6 chickens in the casein group. Pantothenic acid-deficient chickens, although in as poor condition as biotin-deficient chickens, showed perhaps somewhat less severe infections than control chickens on a complete diet. This result indicates that the more severe infections which developed in biotin-deficient animals were specifically associated with the low biotin level of the host rather than with any general weakness produced by the biotin deficiency.

23. *The Coccidial Nature of "Avian Toxoplasma."* FREDERICK COULSTON, University of Chicago.

Nöller (1920) suggested that certain *Toxoplasma* of birds could be coccidial merozoites. Manwell and Goldstein (1938) demonstrated differences in morphology and host infectivity between avian and mammalian types of *Toxoplasma*. Celloidin sections of tissues fixed in Zenker-formalin and stained by the Maximow method, revealed that the so-called *Toxoplasma* of English sparrows, captured in Syracuse, N. Y., was probably the result of a coccidial infection of the intestine having no apparent relationship with classical *Toxoplasma*. Coccidial merozoites were observed in lymphocytes, monocytes and macrophages. They occurred in large numbers in the intestine, spreading via the blood, in which they were observed, to the spleen, liver, lungs, brain and kidney. In these organs, parasites were found localized in small areas.

A detailed study of *Toxoplasma* (Sabin's strain) in mice disclosed no coccidial-like stages. The parasites were observed in lymphocytes, monocytes, macrophages (lymphoid-macrophage system), heterophils and in epithelial cells of many organs where actual destruction occurred. Classical toxoplasmosis in mice was primarily a generalized infection of the subcutaneous tissues while the avian coccidial infection was localized. On a coccidial basis, the infection of the sparrow explained many problems, especially the inability to transmit the parasite from bird to bird. Mammalian type *Toxoplasma* can be easily given to birds by inoculation.

True *Toxoplasma* in birds has been reported in South America and other countries but there is no actual record of it in the U. S. A. If a parasite reservoir exists in northeastern United States, it is probably in some other animal, such as the rat or mouse. Olafson and Monlux (1942) report that sheep, dogs, and cats may be naturally infected in New York State.

24. *Maintenance of Human Toxoplasma in Chicken Embryos.* FRUMA WOLFSON, Johns Hopkins University.

The strain of *Toxoplasma* used was the original "BD" strain isolated from a child in New York City by Wolfe, Cowen and Paige in 1939. The present summary is based on 2 series of chicken eggs, each consisting of (a) 9 consecutive subinoculations and (b) several successive collateral passages. From 2 to 16 eggs were used for each subinoculation. The age of these eggs was from 7 to 13 days. About 20 organisms per egg were found sufficient to produce the infection; the actual numbers used were up to 200. The suspension in physiological salt solution of *Toxoplasma* obtained from the infected chorio-allantoic membrane of a single egg, was inoculated (under the chorio-allantoic membrane) into the succeeding set of eggs. After the inoculation, the eggs were reincubated for 7-9 days before the next subinoculation.

Chicken eggs, which were inoculated with human *Toxoplasma*, developed macroscopic lesions on the chorio-allantoic membranes similar to those previously observed (Wolfson, 1941) in duck eggs inoculated with the guinea pig strain of *Toxoplasma*. These lesions appeared about 5 days or later after inoculation, depending on the size of the inoculum used. The liver and spleen of the infected embryos sometimes were necrotic and contained *Toxoplasma*. The lungs were also found to contain parasites. After seven passages through eggs, *Toxoplasma* produced infections in mice.

It seems of particular importance to note that *Toxoplasma* was consistently found in the erythrocytes of infected embryos.

25. *Minimum Heat Treatment Required for the Destruction of Trichinae in Pork Scraps in Garbage.* WILLARD H. WRIGHT AND JOHN BOZICEVICH, National Institute of Health.

Tests were carried out by introducing into garbage cooked by steam in an open tank pieces of trichinous pork varying in dimensions from $1 \times 1 \times 1$ inch to $6 \times 6 \times 4.5$ inches and in weight between 20 and 2,297 grams. In one series the samples were held in the tank for respective periods of 10, 15, 20 and 30 minutes after the garbage came to a boil. In a second series, the steam was turned off after the garbage had boiled for 30 minutes and the samples were removed 60 minutes later. In the first series considerable variation was noted in the degree of internal heat reached in the pieces of pork and such variation was not always correlated with the size of the sample or the length of cooking. The variation was no doubt due to the fact that the temperature throughout the mass of garbage was not uniform. In the second series, all larvae were killed in all samples with the exception of the largest, which measured $6 \times 5 \times 4$ inches and weighed 2,056 grams. In both series no larvae survived when the internal temperature of the sample reached 56°C . It is concluded that the boiling of garbage for 30 minutes in an open container will effect destruction of trichina larvae in pieces of pork up to 3 inches in thickness and probably in pieces of pork of greater thickness provided the garbage is allowed to cool gradually. Such procedure would thus prevent the transmission of trichina infection to garbage fed hogs.

26. *The Rôle of Parasitologists in World War II.* HENRY E. MELENEY, New York University College of Medicine.
Presidential address.

27. *The Effect of Long Ultraviolet and Near Visible Radiation on the Eggs of the Nematodes, Enterobius vermicularis and Ascaris lumbricoides.* MYRNA F. JONES AND ALEXANDER HOLLAENDER, National Institute of Health.

Eggs of the nematodes, *Enterobius vermicularis* and *Ascaris lumbricoides*, were exposed to high intensities of near ultraviolet and short visible radiation in the range of 3500 to 4900 Å. No ultraviolet shorter than 3500 Å reached the eggs and all infrared radiation was filtered out. The most intense lines were the 3650 Å group of the Hg spectrum; next in intensity was the 4046 Å group; then the 4358 Å group and some minor lines. The intensity approximated 7.5×10^6 ergs/cm²/minute. It was

similar in intensity to this wavelength range in tropical sunlight. These are wavelengths not absorbed by most colorless window glass. Times of exposure varied from 45 minutes to 5 hours. Eggs of *E. vermicularis* were in the infective stage; effect of irradiation was judged by the subsequent ability of active larvae to hatch in artificial gastric juice. Most eggs of *A. lumbricoides* were irradiated in the one-cell stage but some were in a late segmentation stage and a few were embryonated; effect was judged either by ability to complete development or by activity of the vermiform embryo. Lethal effects were observed after irradiation with sufficient energy. Much more energy was necessary to kill at 3500 to 4900 Å than with ultraviolet shorter than 3000 Å. A delay in development of *A. lumbricoides* was apparent after some exposures. Tests with *E. vermicularis* indicated a temperature factor, increasing temperature apparently enhancing the effect of irradiation. These experiments are of interest in connection with numerous reports on the effectiveness of sunlight in destroying nematode eggs, especially eggs of the various ascarids.

28. *Phyllodistomum etheostomae* n. sp. (Trematoda: Gorgoderidae) from Percid Fishes. JACOB H. FISCHTHAL, University of Michigan.

Three of 20 northern greenside darters, *Etheostoma b. blennioides*, collected from the Huron and Saline Rivers, one of 21 blackside darters, *Hadropterus maculatus*, and one of 23 northern logperch, *Percina caprodes semifasciata*, collected from Honey Creek, all in Washtenaw County, Michigan, were found to harbor in their urinary bladders a total of 6 mature and 2 immature specimens of *P. etheostomae*. Average measurements of 5 mature worms: Body 1.766×0.738 mm; oral sucker 0.258×0.228 ; acetabulum 0.188×0.194 ; vitellaria 0.138×0.082 ; ovary 0.146×0.139 ; anterior testis 0.148×0.122 ; posterior testis 0.178×0.129 . Posterior portion of body thrown into two marginal folds. Intestinal crura end at posterior fourth of body; lined with a conspicuous layer of cells. Ovary equal to either testis or larger. Uterus intra- and extracecal. Eggs 0.031×0.022 mm. Testes irregularly lobate; indentations deeper and lobes more numerous than in ovary. The present species most closely resembles *P. brevicecum* and *P. pearsei* from which it is readily distinguished by several characteristics.

29. Increased Mortality in Rats with Concomitant Dual Infections. GRAEME A. CANNING AND JOHN M. FISHER, University of Tennessee.

In order to establish a measure of the fatality of dual infections over single infections, sub-lethal doses of *Trichinella spiralis* were given to white rats and on the same day these were inoculated intraperitoneally with a strain of *Trypanosoma lewisi*. Twenty-two rats thus infected along with controls bearing single infections showed that dually infected rats frequently succumbed to the infection on or about the 14th day following inoculation with *Trypanosoma lewisi* and the feeding of *Trichinella spiralis*.

30. The Effect of Dry-Heating the Ration on Oöcyst Production in *Eimeria nieschulzi* Infections. ELERY R. BECKER, Iowa State College.

Dry-heating either the wheat middlings or the crude casein, or half of the crude casein, in a ration with but these 2 protein-containing ingredients, significantly reduces the number of oöcysts eliminated by rats during *Eimeria nieschulzi* infection. Feeding pantothenate to rats receiving dry-heated crude casein in the ration significantly increases the numbers of oöcysts eliminated. The substitution of vitamin-free casein for dry-heated crude casein produces practically the same effect as dry-heated casein on numbers of oöcysts eliminated, but the rats do not grow so well.

31. Effect of X-Irradiation upon the Resistance of Rats to *Trypanosoma lewisi*. DOROTHY NEUHOF NAIMAN, Hunter College and Columbia University.

Bartonella-free albino rats of different ages were used to determine whether X-irradiation would affect resistance to *Trypanosoma lewisi* infection. The maxi-

mum X-ray dosage which permitted the survival of normal, uninfected rats for 28 days was previously found to be 300, 400, 500, and 500 r, respectively, for 15-, 25-, 35-, and 60-day-old rats. Forty-six rats were employed in the critical experiment, 2 litters being used for each age group. Of each litter, some rats were irradiated only, some infected only, and some both irradiated and infected. Within one hour of exposure, all rats to be infected received from 10,000 to 150,000 trypanosomes in blood-broth suspension, intraperitoneally, all the rats of each age group receiving the same dose. The course of the subsequent infection was followed by counting the number of trypanosomes in the circulating blood at frequent intervals, in most cases on alternate days. Rat weights were recorded and leucocyte counts made at the same time, and blood smears were taken for subsequent estimation of the proportion of dividing parasites. It was found that the peak of the infection was significantly higher and the infection lasted longer in the irradiated members of each series than in the control rats. The only deaths which occurred were in the irradiated groups. Thus it appears that X-irradiation lowers the resistance of rats to *T. lewisi* infection.

32. *Number of Larvae and Time Required to Produce Active Immunity in Rats against Trichinella spiralis.* JACOB H. FISCHTHAL, State University of Iowa.

Fourteen of 16 rats given either 80, 160, 320 or 640 larvae of *Trichinella spiralis* and a test dose of 10,000 larvae 7 days after the initial infection died between the third and eighth days after reinfection. Between 2,592 and 6,828 developing larvae were recovered from their intestines, indicating that no immunity is produced within 7 days after feeding 80 to 640 larvae. The two similarly infected rats which survived the test dose had in some way been able to resist the effects of the latter. On the other hand, 15 of 16 rats given either 80, 160, 320 or 640 larvae and a test dose of 10,000 larvae 14 days after the initial feeding survived. Nine of these were killed 7 days after reinfection, and only 1 to 546 developing larvae were recovered from their intestines. Seven of the 9 rats contained less than 33 adults. Definite immunity is herein demonstrated since non-immunized control rats harbored approximately 33 per cent of a 10,000 dose. The numbers of larvae recovered from the muscles of the remaining 7 rats similarly infected showed that immunity had been produced by the initial infection and that the rats were nearly perfectly protected against muscle invasion. Therefore, a single small dose of larvae is capable of producing immunity and a period of approximately 14 days is adequate to develop this immunity. It is noteworthy that after immunization by infection the immunity is effective against the intestinal phase of the parasite.

33. *Trichinella Skin Tests in Apparently Normal Individuals.* HARRY M. ROSE AND JAMES T. CULBERTSON, Columbia University.

In a preliminary study *Trichinella* skin tests were performed on 232 second-year medical and dental students, most of whom were males from 22 to 26 years of age. The tests were carried out by injecting 0.1 cc of a 1-1000 dilution of *Trichinella* antigen intradermally, and recording the results at the end of 15 minutes. Reactions were considered to be positive when an urticarial wheal at least 1.0 centimeter in diameter appeared at the site of injection.

Four groups of individuals from two schools were tested. In Group I (School A), 5 of 48 persons were positive (10.4 per cent); in Group II (School A), 2 of 17 were positive (11.7 per cent); in Group III (School A), 9 of 101 were positive (8.9 per cent); in Group IV (School B), 3 of 66 were positive (4.5 per cent). Thus, the incidence of positive skin reactions varied from 4.5 per cent to 11.7 per cent among the groups, with an over-all incidence of 8.2 per cent of positive reactions.

Precipitin tests were also performed with sera from eleven of the nineteen positive individuals, of which five were positive and six were negative.

The results of this study are in accord with those of other investigators, and indicate that a significant proportion of young, apparently healthy individuals have

endured a sub-clinical infection with *Trichinella spiralis*. The need for a survey of the comparative efficiency of the intradermal test, the precipitin test, and the complement fixation reaction in trichiniasis is suggested.

34. *Immunological Studies on a Polysaccharide and Protein Fraction Isolated from Trichinella spiralis*. LEO R. MELCHER, University of Chicago.

Polysaccharide and protein antigens were isolated from larvae of *Trichinella spiralis*. These were used for skin testing and serological studies of *Trichinella*-infected rabbits. The polysaccharide was precipitinogenic in high dilutions but did not act as a skin-test antigen except when large amounts were administered (0.5–1.0 mgm). Cross reactions with antisera against other roundworms such as *Ascaris suum*, *Nippostrongylus muris* or the larval tapeworm, *Cysticercus taeniaeformis* did not occur with this antigen. This nondialyzable polysaccharide gave a Molisch reaction in extremely high dilutions, furthermore protein tests were negative in relatively high concentrations, and it was nitrogen-free when tested by Nessler's method on a 10-mgm sample.

A protein fraction isolated by alkali extraction of the whole worm material and purified by acid precipitation of nonreactive substances was both precipitinogenic and induced positive skin reactions in high dilutions. This protein fraction was studied in the Tiselius apparatus and its electrophoretic pattern consisted of three components with different mobilities.

35. *Active Immunization of Sheep against Large Single Test Infections of Haemonchus contortus*. NORMAN R. STOLL, Rockefeller Institute for Medical Research.

Sheep have been protected against large test infections of *Haemonchus contortus* by the parenteral injection of sterilized, exsheathed infective larvae of the same species. Such injected larvae do not wander to the abomasum, but instead induce a state of insusceptibility which may be highly effective against larvae normally inoculated per os in single doses of size sufficient to kill clean controls. Positive results have been secured with a single intraperitoneal or subcutaneous injection made 0 to 11 weeks before test. The studies have re-emphasized also the potency of the protection against *Haemonchus contortus* afforded this host when it is well immunized. Experiments were conducted in the fall and winter with spring lambs reared helminth-free.

36. *Some Preliminary Experiments on a Skin Test for Immunity to the Stomach Worm Haemonchus Contortus in the Calf*. ROY L. MAYHEW, Louisiana State University.

Preliminary experiments indicate that injection of a saline extract of adult worms produces a positive reaction, indicated by a swelling about the point of injection, in immune animals. No reaction was produced in a nonresistant and an infected animal.

37. *Acquired Resistance to the Gapeworm Syngamus trachea in the Turkey and Ring-necked Pheasant*. LOUIS OLIVIER, United States Bureau of Animal Industry.

Each of fifteen 49-day-old turkeys received an initial infection of *Syngamus trachea* by swallowing about 10,000 embryonated eggs and larvae. Forty-one days later 7 birds harbored an average of 23 pairs of worms each. Each of the remaining 8 birds swallowed about 5,000 additional eggs and larvae. Fifteen days later each superinfected bird harbored from 0 to 9 pairs (average 2.3) of worms from the first infection as determined by size of worms and lesions; no worms from the second infection were present. Eight previously uninfected controls, fed 5,000 eggs and larvae, harbored 39 to 168 pairs (average 108).

Each of twenty-two 39-day-old pheasants received an initial infection by swallowing about 3,000 eggs and larvae. Forty-one days later 5 birds had lost their worms, except 2 living male worms from which the females had become detached

and lost, and 2 pairs of dead worms. Each of the remaining 17 birds received about 5,300 additional eggs and larvae. Thirteen days later each superinfected bird harbored from 0 to 4 pairs (average 0.7) from the second infection and none from the primary infection. Five previously uninfected controls, fed 5,300 eggs and larvae, harbored 25 to 177 pairs (average 94).

It appears, therefore, that resistance to reinfection with *Syngamus* does not depend upon the presence of large numbers of worms in the trachea at time of reinfection. Moreover, the results of the experiment with pheasants suggest that the resistance persists after the worms have been lost.

38. *Skin Tests in Schistosomiasis Mansoni Patients with Antigen from Pneumonoecus medioplexus.* JAMES T. CULBERTSON AND HARRY M. ROSE, Columbia University.

Three patients with proved schistosomiasis mansoni were skin-tested with 0.1 cc of a one per cent extract of powdered *Pneumonoecus medioplexus*. All manifested an immediate reaction, consisting of a wheal 1.5 to 3.0 centimeters in diameter with distinct pseudopodia. Twelve persons not infected with schistosomes manifested no reaction when tested with the same extract. Further tests in the patients showed that the one per cent extract could be diluted at least two hundred times yet elicit a skin response. The extract resisted boiling for 10 minutes, and retained most of its activity after being autoclaved. The substance responsible for the reaction was soluble in water, but almost wholly insoluble in alcohol or ether.

Four persons in whom schistosomiasis mansoni had been successfully treated with antimony compounds, respectively, two and one-half, three, four, and six years earlier, were also skin tested. A very slight skin reaction was seen in the patient treated two and one-half years previously. No skin reaction was manifested by the three remaining persons.

39. *Resistance to Ascaris lumbricoides L. in Guinea Pigs and the Eosinophilia Associated with Infection.* A. MURRAY FALLIS, Ontario Research Foundation.

Infection with *Ascaris lumbricoides* L. in guinea pigs has been studied in a series of experiments using over three hundred animals. The results confirm and extend those of Kerr and others who have studied this parasite. Heavy infection with *Ascaris lumbricoides* renders guinea pigs almost completely resistant to this parasite for a short time immediately following the infection and partially resistant for at least 15 weeks. The resistance appeared to be more complete in pigs which had previously received three heavy infections at intervals of 10 days or less. Evidence of this resistance was obtained by noting the clinical symptoms produced, the rate of growth of the parasite larvae in resistant versus control animals and the pathology of the liver and lungs in each (the latter was illustrated by Kodachrome pictures), and the number of larvae recovered from the lungs after digestion. Attempts to produce passive resistance by injections of serum from resistant pigs met with limited success only. Heavy infection was accompanied by an eosinophilia which reached a peak in 7-10 days, then declined gradually to normal. The eosinophilia produced in resistant and control pigs has also been followed and compared. Guinea pigs receiving a sublethal infection lost weight from approximately the sixth to eleventh day, after which they began to gain. No marked temperature change was observed in pigs receiving a sublethal infection.

40. *The Relation of Splenectomy and the Resistance of Old Mice to the Mouse Cestode, Hymenolepis nana var. fraterna.* JOHN E. LARSH, JR., Johns Hopkins University.

In connection with studies on the mouse cestode, *Hymenolepis nana* var. *fraterna*, experiments were undertaken to determine the influence of the spleen on natural resistance. Sixty mice (two months old) were selected for an experiment, and 30 were splenectomized. One week after splenectomy, 15 were tested and had about the same percentage development of cysticercoids (about five) as the con-

controls. However, five months later when the other 15 splenectomized mice and controls were infected, there was considerable difference in the percentage development. The controls harbored only a few parasites (0.5 per cent development), whereas large numbers were observed in the splenectomized animals (a 4.5 per cent development). The total number of cysticercoids present in the latter was just slightly less than that in young mice two and one-half months old (included as a check on the viability of the eggs used). Similar results were obtained when the experiment was repeated. The low percentage development in the old controls (seven months old) is a phenomenon which has been observed previously in old mice. In the splenectomized group, latent *Bartonella* infections were manifested three days after removal of the spleen. Periodic hemoglobin (Sahli) determinations showed a gradual development of anemia, probably caused by the *Bartonella*. The hemoglobin level decreased from 101 per cent before the operation to less than 80 five months later, while it remained practically unchanged in the controls. As the anemia progressed, there was a higher percentage development of cysticercoids. Thus this anemic condition seems to be the most plausible explanation for the failure of these mice to develop resistance.

41. *The Effect of Ascaris suum Extract Injections upon Chickens Infected with Ascaridia lineata.* L. L. EISENBRANDT, University of Kansas City.

Four subcutaneous injections of dried extract of *Ascaris suum* suspended in physiological salt solution were given in increasing doses on alternate days to half grown white leghorn chickens. The doses were 12, 14, 31 and 56 mg protein. Four days after the last injection 50 ± 5 infective eggs of *Ascaridia lineata* were administered, and three weeks later the chickens were killed and the number and length of *A. lineata* were determined. The control chickens were infected as above.

The injections of *A. suum* extract failed to increase the resistance of these chickens against *A. lineata*. The resistance appeared to be lessened because the experimental chickens averaged 5.3 more nematodes than the control chickens (statistically significant), and the nematodes were longer (2.36 mm, but this was not significant) than those in the controls. Therefore, in this experiment, injections of *A. suum* extract actually lowered the resistance of chickens to *A. lineata* rather than increased it. This study was possible by aid from Sigma Xi research grant and from Dr. J. E. Ackert.

42. *Effects of the Nematode, Trichostrongylus colubriformis, on the Nutrition of Lambs.* JOHN S. ANDREWS, W. R. KAUFFMAN AND R. E. DAVIS, United States Bureau of Animal Industry.

The nutrition of the six cross-bred (Hampshire-Southdown) castrated male lambs, of which five were experimentally infected with from 63,000 to 440,000 infective larvae of *Trichostrongylus colubriformis* each, was studied experimentally. One lamb served as an uninfected control for 14 of the 18 weeks during which the infected lambs were under observation. The presence of large numbers of mature trichostrongyles in the infected lambs, indicated by the passage of approximately 1,500,000 worm eggs per 24 hours, for more than a month, apparently did not decrease the digestibility coefficients of any constituent of the feed or interfere with the absorption of calcium and phosphorus except as noted below. In one lamb showing clinical symptoms of trichostrongylosis, and later dying of the disease, the digestibility coefficients of all components of the feed, except ether extract and crude fiber, were decreased when the vitality of the host had been so reduced by partial starvation, and dehydration through continuous diarrhea, that normal digestion and absorption could no longer take place. One lamb that had suffered from anorexia and diarrhea for approximately seven and one-half weeks as a result of *Trichostrongylus* infection lost from the skeleton calcium and phosphorus necessary for the normal metabolism, as shown by analysis of the right femur. The energy metabolism of the infected lambs and the number of pounds of feed necessary for

them to produce 100 pounds of live weight were found to be about twice that required by the uninfected control lambs.

43. *Symptoms and Immunity following Graduated Doses of Eimeria tenella.* HARRY A. JANKIEWICZ, University of Southern California.

Groups of chicks were administered respectively 50, 250, 500, 1,000, 3,000, 6,000, 20,000, and 100,000 oöcysts of *Eimeria tenella*. By employing a sugar solution of specific gravity of 1.065, it was possible to make accurate counts of oöcysts which were uniformly distributed. Daily weight gains, hemorrhage, severity of symptoms, mortality, and the speed of convalescence were the criteria to judge the severity of infection. The chicks fed 50 oöcysts were not harmed. Those fed from 250 to 1,000 oöcysts experienced, respectively, mild to moderate symptoms but no mortality. Weight losses increased as the dosage was raised from 1,000 to 100,000, the latter dosage causing a loss in weight equal to that gained by the controls. Hemorrhage increased as the dosage was raised from 250 to 100,000. Mortalities increased as the dosage was raised from 3,000 to 100,000 oöcysts. Convalescence was more rapid following dosages as high as 6,000 than following those above 20,000. A dosage of 100,000 oöcysts given to each chick eight days after the initial inoculations formed the basis of the immunity rating, the latter being placed on a percentage basis. About a 10 per cent average immunity rating was acquired from 50 oöcysts; a 30 per cent average rating from 250 oöcysts; a 50 per cent rating from 500 oöcysts; 70 per cent ratings from 1,000 to 3,000 oöcysts; and about a 90 per cent average immunity rating from 6,000 to 100,000 oöcysts. Dosage determined the severity of cecal coccidiosis and the average level of immunity developed.

44. *Studies on Susceptibility of Chickens to Cecal Coccidiosis.* C. A. HERRICK AND S. A. EDGAR, University of Wisconsin.

When sporulated oöcysts of *Eimeria tenella* were administered to chickens which had been off feed until their crops were empty, the oöcysts reached the cecal pouches in large numbers in less than an hour. But when chickens with full crops were infected with comparable doses, the oöcysts reached the cecal pouches in two and one-half to four hours. Groups of chickens kept under carefully controlled laboratory conditions were always more susceptible to coccidiosis if infected when their crops were empty. From two to eight times as many chickens died from coccidiosis among those groups infected when their crops were empty as compared with those infected when their crops were filled.

Similar results were obtained when chickens were kept under range conditions. Several groups of chickens were so alternated in naturally contaminated brooder houses that they had identical conditions except that some had feed available when they left the hover in the morning while others were not fed until 8 A.M. In those groups which were not fed until 8 A.M. three times as many died of coccidiosis.

45. *The Magnitude of the Hookworm Problem in a Typical County of Southern Georgia.* J. ALLEN SCOTT AND JUSTIN ANDREWS, Georgia Department of Public Health.

In recent years, differing opinions have been expressed with regard to the present magnitude of the hookworm problem in the southern United States. It is known that the worst areas are in the pine woods sections of the sandy coastal plain. Here the people derive a meager subsistence from cropping small fields and gathering turpentine. Sanitation is virtually non-existent, and diets are extremely inadequate. By means of a house-to-house collection of fecal specimens and determinations of hemoglobins, quantitative data are now available on a cross section of the population of a typical county in this region. Two-thirds of the group between five and twenty years of age were found to be infected, half were passing more than 5,000 eggs per cc (basis formed stools), and 10 per cent were passing more than 40,000 eggs per cc. This latter 10 per cent showed an average hemoglobin of 5 grams as compared with

an average 10.5 grams for non-infected persons in the same family. The problem is only slightly less in younger children. In older persons the prevalence is also high, but the disease only occasionally severe. Statistical methods have been developed to make possible a simple expression of the significance of this problem in terms which can be understood by the layman. For the first time an attempt has been made to visualize the problem on a family basis. It is believed that the information is a step toward more accurate evaluation of the hookworm problem of the South.

46. *Use of Calcium Chloride to Isolate Helminth Ova from Soil.* R. T. STEVENSON, University of Wisconsin.

Attempts to recover *Toxocara canis* ova from artificially infected soil samples using recommended methods and flotation media gave erratic results. The possibility of soil particles adhering to the eggs suggested the use of solutions of greater specific gravity than are normally employed. CaCl_2 solution (sp. gr. 1.35-1.40) gave consistently better results than ZnSO_4 of the same specific gravity or NaCl (sp. gr. 1.20) solution. A comparison of the Willis flotation method and the Lane direct centrifugal flotation technique using CaCl_2 solution (sp. gr. 1.35) was made and results showed the latter to be more efficient. Soaking soil samples in 1 per cent solution of CaCl_2 over night or in a stronger (10 per cent) solution for at least an hour before attempting to float out the eggs provided even better results. After soaking the sample it was screened to remove coarse debris. The Lane tube containing the sample was attached to a vacuum pump to remove air trapped in the soil. These last two procedures greatly facilitated microscopic examination. Prolonged exposure to the concentrated solution does not appear to destroy ova of *T. canis*, *Eucoleus aerophilus*, *Capillaria plica* or coccidia oöcysts but hookworm eggs disintegrate in it rather rapidly.

47. *Phenothiazine in the Treatment of Enterobiasis.* ELLA KUITUNEN, University of Toronto.

Purified phenothiazine has been used in the treatment of enterobiasis in over 300 children and adults. In approximately 95 per cent of the whole group repeated swabs have failed to show the presence of pinworm infection after one course of treatment covering 5 to 6 days. The NIH swab was used for the detection of infected cases as well as for the post-treatment re-examination. Five swabs on consecutive mornings were taken for diagnosis. The post-treatment swabs were taken on 7 consecutive mornings commencing one week after the last dose of phenothiazine. No laxative was given before, after or during the treatment. Phenothiazine was usually given mixed with food or orange juice.

The following dosage has been used:

Age groups	Total dosage in grams	Daily dose in grams
2-5	5	1
6-8	6	1
9-11	7.5	1-1.5
12-adults	9	1.5

48. *The Efficacy of Phenothiazine and Nicotine-Bentonite for the Removal of Heterakis gallinae and Ascaridia galli from Chickens.* JAMES E. GUTHRIE AND PAUL D. HARWOOD, Dr. Hess and Clark Laboratories.

Tablets manufactured from 33 parts phenothiazine, 66 parts nicotine-bentonite (5 per cent nicotine), and 1 part sodium stearate weighed on the average 1.33 grams ($s=0.029$). Of 16 brown leghorn cockerels, 4 were treated with these tablets, 4 with equivalent amounts of phenothiazine, 4 with equivalent amounts of nicotine-bentonite and 4 were used as untreated controls. The birds receiving tablets of phenothiazine and nicotine-bentonite eliminated 83.7 per cent of 1012 *Heterakis* and 96.2 per cent of 131 *Ascaridia*; those receiving phenothiazine, 89.9 per cent of 675 *Heterakis* and 48.2 per cent of 110 *Ascaridia*; those receiving nicotine-bentonite,

10.1 per cent of 1246 *Heterakis* and 85.2 per cent of 149 *Ascaridia*; those receiving no treatment, 1.1 per cent of 1145 *Heterakis* and 8 per cent of 137 *Ascaridia*. To estimate the toxicity of these tablets, 23 birds weighing from 970 to 1,500 grams were given from 5 to 40 tablets each. All birds survived, but those receiving 20 or more tablets lost weight. To estimate the effect of extreme fineness on the efficacy of phenothiazine, 10 pairs of birds were matched by weight. One bird of each pair was given 150 milligrams of micronized phenothiazine (specially ground as fine as possible) the other was given 150 milligrams of commercial phenothiazine (about 90 per cent of this phenothiazine passes through a screen of 100 meshes to the inch). Birds receiving micronized phenothiazine eliminated 47.9 per cent of 879 *Heterakis*; those receiving commercial phenothiazine eliminated 68.2 per cent of 786 *Heterakis*.

49. *Observations on the Life History of a Rabbit Cuterebrid, the Larvae of Which May Penetrate the Human Skin.* RAYMOND H. BEAMER, University of Kansas, AND LAWRENCE R. PENNER, University of Connecticut.

A large, white-sided female cuterebrid collected in a pasture near Lawrence, Kansas, on July 10, 1942, laid numerous eggs in rows up to three-fourths of an inch long on wild lettuce and the sides of a laboratory cage. Larvae started to hatch about 8:00 AM C. W. T. on July 14 and continued hatching for several hours. Three larvae were allowed to penetrate the human skin. Complete entry of one larva took 18 minutes and the other larvae were two-thirds in when removed at 18 and 25 minutes from the time of first attachment. Larvae placed on two guinea pigs, four white rats, one white-footed mouse, and four pack rats, failed to mature warbles. Twenty-two larvae were placed on a half-grown, white rabbit, four of which were inserted into the nostrils. The rabbit died July 20 and a single second instar larva was found under each foreleg in connective tissue between the skin and underlying muscle tissue. One had made an opening in the skin to the exterior. Eight larvae placed on two similar rabbits matured three and one larvae by August 13, although the existence of four and five had been noted previously. Apparently three of the mature warbles were eaten by the rabbits; the fourth pupated August 14. Very rapid healing of the empty warble holes was noted.

50. *On the Structure of the So-Called "Stigmata" of Larval Ticks.* HAROLD ELISHWITZ, University of Minnesota.

Despite assertions by morphologists that larval ticks lack a tracheal system, tick taxonomists have, almost without exception, continued to refer to a series of four pairs of placodes in the body wall as "stigmata." These placodes, appearing in surface view like a pair of mitral valves surrounded by a sclerotized peritreme, are located posterior to the subcoxae of the three pairs of legs on the ventral surface and anterior to the last festoons on the dorsolateral surface. The distribution of these plaques shows generic and specific variation.

It is here shown that each of these "stigmata" is in reality the orifice of a complex sensory-glandular system to which the term sensillum glandularis is applied. The system, in *Dermacentor variabilis*, consists of an elliptical, cone-shaped sclerotized annulus surrounding a bullet-shaped, thin-walled, hollow sensory cone. The apical quarter of the cone is invested by a chitinous apical membrane that arises from a small membranophore ridge which rings the inner wall of the annulus. The base of the cone sits upon an elliptical sensillar ring into whose hollow center a solid sclerotized sensory peg is inserted. Two closely apposed, relatively enormous hypodermal gland cells pour their secretion into the space between the cone and the annulus. Located between the gland cells is a prominent neurone whose efferent fibers terminate at the base of the sensillar ring and sensory peg. The glands function during molting while the sensory function is probably olfactory.

51. *The Taxonomy of the Pathogenic Rickettsiae.* CORNELIUS B. PHILIP, Army Medical School.

In reviewing the purely systematic aspects of the human pathogenic rickettsiae, it is pointed out that considerable confusion has arisen from disregard of accepted taxonomic procedure in proposal of many new specific names. Adequate taxonomic stability will have to await further "biological" clarification of the interrelationships of the aetiological agents now arbitrarily placed in the group. Many synonyms are apparent even now, however, among specific names. Excluding certain problematic forms in this discussion, the accepted species may be grouped under 3 subgenera of the genus *Rickettsia*, namely, *Rickettsia* s. str. which includes the type species *provaszeki* (with 1 synonym) of epidemic typhus, subspecies *typhi* (4 synonyms including *mooseri*) of endemic typhus, and *orientalis* of tsutsugamushi fever (8 synonyms); *Dermacentroxenus* which includes *rickettsi* (2 synonyms) of Rocky Mountain spotted fever and subspecies *conori* (2 synonyms) of boutonneuse fever and South African tick bite fever; and a new subgenus for the Q fever group with one species, *burneti* (1 synonym).

52. *Age as a Factor in the Resistance of Splenectomized Rats to Infection with Bartonella muris.* WALTER R. KESSLER, Columbia University and College of the City of New York.

Splenectomized rats of six different age groups (ranging from 12 to 150 days) were inoculated with various concentrations of *Bartonella muris*-infected erythrocytes. Inocula which induce fatal infection in almost 100 per cent of the older animals tested (27, 40, 60, 80, and 150 days of age) produce no fatalities among rats of suckling age (12 days at the time of inoculation). Not only the eventual outcome of the infection but the development, course, and symptomatology of the disease in animals of suckling age differs from that shown by other age groups. Although the patent period was found to be fairly constant for all animals, being on the average about 3 days, the prepatent period was longest for rats of suckling age. Invariably, in rats of the older age groups, a marked loss in body weight is observed during the terminal phase of the infection and hemoglobinuria is common. In none of the members of the 12-day-old group were either weight loss or hemoglobinuria noted. In older animals as many as 80 or 85 per cent of the red cells may be invaded by numerous (40 to 50) parasites. On the other hand, in rats of suckling age, both the number of infected cells as well as the number of organisms per cell is considerably less; indeed, only in rare instances are as many as 50 per cent of the erythrocytes invaded.

The general level of red cell production as indicated by the presence of immature erythrocytes, particularly normoblasts, was more vigorous in the rats of suckling age than in older animals. Normoblasts appear to be completely resistant to invasion by *Bartonella muris*; immature red cells likewise tend to be less frequently and more sparsely infected. This fact is no doubt directly related to the relative resistance of splenectomized rats of suckling age to infection with *Bartonella muris*.

53. *The Pathogenicity of Three Strains of Trichinella spiralis as Indicated by Lethal Dose and Survival Time.* IRVING RAPPAPORT, Cornell University.

Although some workers believe that a variation in virulence may exist among strains of *Trichinella*, no evidence for it has been reported. In the present study of the problem, larvae of three different strains were obtained by peptic digestion, counted directly in graded doses, and administered to mice by stomach tube. Results obtained with the three strains were quite similar in regard to lethal dose and survival time. By combining the results obtained with all three strains, these criteria of lethal dose and survival time can be more accurately evaluated.

All animals receiving overwhelming doses (over 140 larvae per gram of body weight) died between 2 and 4 days after infection. The survival time up to 60 days was quite variable when doses between 60 and 100 larvae per gram were given, making it almost impossible to predict the survival time. The percentage of animals which survived was dependent upon dosage. All mice receiving more than 80 larvae

per gram died during the 60-day interval. The percentages of animals that survived doses from 10 to 85 larvae per gram of body weight at 10 larvae per gram intervals were: 100, 100, 64, 65, 44, 30, 5, and 5, respectively. There appeared to be little or no difference among the three strains of *Trichinella* studied. It is believed, however, that the criteria of lethal dose and survival time possess many limitations and that other methods, such as longevity and sex-ratio studies of the adult parasites, are far superior for such investigations in experimental trichiniasis.

54. *Tests for a Hemolytic Substance in Chabertia ovina.* W. L. THRELKELD, Virginia Agricultural Experiment Station.

Ten thousand infective larvae of *Chabertia ovina* were administered to a three-month-old lamb. The animal was killed two months later, and 426 mature *Chabertia* were recovered. Two hundred and fifty worms, fixed in 70 per cent alcohol, were utilized as described. The anterior portion of the worms (esophagus and head) was severed. The anterior and posterior portions were washed repeatedly in Ringer's solution, then in 0.87 per cent saline, transferred to 95 per cent alcohol, triturated, and filtered through a Berkefeld N filter. After evaporation and drying, the product from the filtrate and the unfiltered residue were taken up in sterile 0.87 per cent saline and added to a solution of fresh defibrinated sheep blood, made by adding 19 cc of sterile 0.87 per cent saline to 1 cc of blood. After 18 hours of incubation at 37.5° C, hemolysis occurred. Similar results were obtained after 22 hours' incubation at 37.5° C from the product of 56 whole worms which were taken from a nine months' old lamb which had retained the infection for five months. A further study is planned to determine more conclusively whether a hemolytic substance is present in *Chabertia ovina*.

55. *Factors Influencing the Oxygen Consumption of a Larval Eustrongylides.* THEODOR VON BRAND, Catholic University of America.

The oxygen consumption of a larval *Eustrongylides* from *Fundulus* remained constant in the pH range of 3.4 to 8.3, but was increased in the pH ranges 1.1 to 2 and 9 to 10.7. The significance of the increased respiration in the latter case remains unclear. The stimulation in the extreme acid range, however, may have a biological foundation, since the worm bores into the stomach wall of the definitive host. The temperature relationships were investigated in the range of 5 to 48° C. The increase in respiration with increasing temperature could be expressed with two lines only if the Q_{10} was calculated or if Arrhenius' formula was used. The influence of various ions in isotonic solutions upon the oxygen consumption was also studied. The least stimulating cations and anions respectively were Na and Cl. The maximally stimulating ones were K and PO₄.

56. *Survival on Soil of Oöcysts of Two Species of Swine Coccidia, Eimeria deblickei and E. scabra.* JOHN LAWRENCE AVERY, United States Bureau of Animal Industry.

During the interval from May, 1941, to July, 1942, the survival periods on soil of oöcysts of *Eimeria deblickei* and *E. scabra* were investigated at Beltsville, Maryland. It was considered that the information obtained would be useful in formulating control measures against these parasites. Two test plots, each 15 feet square, were fenced off in a pasture which for several months previously had been occupied by swine infected with the species of coccidia named. The infected hogs were removed from the area prior to the beginning of the experiment. One plot was constantly shaded by trees, the other was unshaded and both were kept free of vegetation. At intervals of 4 to 8 weeks the viability of the oöcysts was tested by maintaining on each plot for a period of 3 days a pig that had been raised free of coccidia. The pigs were then removed to separate cement-floored pens and the feces collected daily; the numbers of oöcysts present were estimated by the dilution technique. The investigation showed that the oöcysts on both plots were still viable after 15

months. However, infections acquired from the unshaded plot were lighter than those acquired from the shaded area. Temperatures of the surface soil of the two plots ranged from $+40^{\circ}\text{C}$ to -4.5°C . These findings show that under the temperature conditions named allowing an infested hog lot to remain vacant as long as 15 months may not preclude infections of susceptible pigs with the two species of coccidia named and indicate that longer periods of rotation may be essential to effective control.

57. *The Effect of Moderately Low Temperatures on the Sporulation of Oöcysts of Two Species of Swine Coccidia.* JOHN LAWRENCE AVERY, United States Bureau of Animal Industry.

Unsporulated oöcysts of *Eimeria deblickei* and *E. scabra* cultured in tap water were exposed to (1) a temperature slightly above freezing (6 to 8°C), (2) alternate freezing and thawing (0.5 to -3°C), and (3) continuous freezing (-2 to -7°C) to ascertain the effect on sporulation; two cultures were maintained at each temperature; two other cultures kept at room temperature (19 to 25°C) served as controls. Each culture consisted of approximately equal numbers of oöcysts of each of the two species in question in 25 cc of tap water contained in a petri dish 3 inches in diameter; the oöcysts were obtained by the sugar-flotation technique from the feces of a pig harboring a natural infection of the species named. After the cultures had been exposed to the various temperatures in question for a period of 26 days, 100 oöcysts from the two cultures kept at each temperature were examined and the percentage of sporulation ascertained. At that time 93 per cent of the oöcysts in the control cultures had sporulated but no sporulation was observed in any of the cultures exposed to lower temperatures. The cultures were then maintained at room temperature (19 to 26°C) for a period of 14 days and were then examined. The percentages of sporulation in the three groups of cultures at that time were 88 per cent, 67 per cent, and 55 per cent, respectively; no change was noted in the percentage of sporulated oöcysts in the control cultures. In the case of individual cultures approximately equal numbers of oöcysts of the two species sporulated.

58. *The Segmental Anatomy of Mesocostoides variabilis Mueller, 1928, from Didelphis virginiana Kerr.* ELON E. BYRD, University of Georgia, AND JAMES W. WARD, Mississippi State College.

From a study of the immature, mature and gravid segments of a series of specimens taken from *Didelphis virginiana* in Mississippi the writers have been able to definitely identify the tapeworm parasite as *Mesocostoides variabilis* Mueller, 1928. Since no scoleces were recovered from the host, only segments have been examined. These reveal certain features of the anatomy of this cestode which heretofore have remained obscure, or have not been made clear in the published accounts of the species described as belonging to the genus. The arrangement of the muscle fibers, the excretory ducts, the general arrangement of the reproductive organs, and the manner in which the egg capsule (paruterine organ) is formed are some of the features.

59. *Notes on the Genital System of the Bird Fluke, Apharyngostrigea cornu (Zeder).* ELON E. BYRD, University of Georgia, AND JAMES W. WARD, Mississippi State College.

The general anatomy of the bird fluke, *Apharyngostrigea cornu* (Zeder), from the little blue heron, *Florida caerulea caerulea*, from Mississippi, is discussed. From a study of serial sections of the parasite the course and the connections made by the various genital ducts have been worked out in detail.

60. *The Parasites of the Caecilidae (Amphibia: Apoda).* A. C. WALTON, Knox College.

Annotated records are presented from six species of hosts (*Dermophis mexi-*

canus, *Scolecormorphus uluguruensis*, *S. unicolor*, *S. vittatus*, *Siphonops annulatus*, and *Uraeotyphlus oxyurus*) of the presence of six species of Nematoda (*Ascaris unguiculata*, *Oxysomatium mexicanum*, *Railletnema loveridgei*, *R. multipapillata*, *Rhabdias bdellophis*, and *R. escheri*); and from two species of hosts (*Dermophis mexicanus*, and *Uraeotyphlus oxyurus*) of the presence of Trematoda (*Cercorchis patonianus* and *Gorgoderina carli*). Other types of parasites apparently have not been recorded. Brazil, East Africa, India, and Mexico cover the geographical area represented by the various hosts.

61. *The Parasites of the Cryptobranchoidea (Amphibia: Caudata)*. A. C. WALTON, Knox College.

Records of parasitic infection of members of the Cryptobranchoidea are as follows:

I. Hynobiidae: a. *Hynobius fuscus*, *hida*, *kimurae*, *stejnegeri* and *tokyoensis* are infected by *Aplectana* (1), *Rhabdias* (1), *Pharyngodon* (2) and *Capillaria* (1), species of Nematoda; *Hynobius fuscus*, *stejnegeri* and *tokyoensis* by *Gorgoderina* (1) and *Mesocoelium* (2), species of Trematoda; and *Hynobius tokyoensis* by the protozoon *Trichodina okazimae*. b. *Onychodactylus (japonicus)* is infected by *Capillaria* (1), *Pharyngodon* (2), and *Rhabdias* (1), species of Nematoda; and by unidentified flagellates.

II. Cryptobranchidae: a. *Cryptobranchus (alleganiensis)* is infected by *Filaria* (1), *Spirotrax* (1), and *Zanclophorus* (1), species of Nematoda; by *Cercorchis cryptobranchi* (Trematoda); by *Ophiotaenia cryptobranchi* (Cestoda); and by *Proteromonas* (1), *Trichomonas* (1), and by *Trypanosoma* (1), species of Protozoa. b. *Megalobatrachus (japonicus)* is infected by *Filaria cingula* (Nematoda); by *Diplodiscus* (1), *Liolope* (1), *Opisthodiscus* (1), and larval *Phyllodistomum* (1), species of Trematoda; by *Bothriocephalus* (Cestoda); and by unidentified flagellates.

62. *The Parasites of the Ambystomoidea (Amphibia: Caudata)*. A. C. WALTON, Knox College.

Annotated records of the various parasites listed as attacking members of the Ambystomoidea are presented as follows: Ambystomidae: a. *Ambystoma (opacum, talpoideum, texanum, tigrinum, and A. sp?)* by *Capillaria* (1), *Cosmocercoides* (1), *Filaria* (1), *Gordius* (1), *Hedruris* (1), *Spirotrax* (2), and *Spiroptera* (1), species of Nematoda; *Ambystoma (jeffersonianum, maculatum, opacum, punctatum, texanum, tigrinum, and larval ambystomids)* by *Brachycoelium* (1), *Cercaria* (1), *Diplostomulum* (1), *Gorgoderina* (1), *Gorgoderina* (1), *Megalodiscus* (1), *Opisthoglyphe* (1), and *Phyllodistomum* (1), species of Trematoda; *Ambystoma (opacum, and tigrinum)* by *Diphyllbothrium* (1), *Ligula* (1), and *Ophiotaenia* (1) species, and proteocephalid cysts—Cestoda; *Ambystoma opacum* by *Acanthocephalus acutulus* (Acanthocephala); *Ambystoma (jeffersonianum, maculatum, opacum, texanum, tigrinum, and unidentified ambystomids)* by *Balanitidium* (1), *Chilomastix* (1), *Cryptobia* (1), *Cytamoeba* (1), *Eimeria* (1), *Euglenamorphia* (1), *Eutrichomastix* (1), *Glaucoma* (1), *Haptophrya* (1), *Hexamastix* (1), *Hexamita* (1), *Karotomorphia* (3), *Proteromonas* (1), *Protoopalina* (1), and *Trichomonas* (1), species of Protozoa; and *Ambystoma (maculatum and opacum)* by *Hannemania dunni* (Acarina). b. *Dicampton ensatus* larvae by *Phyllodistomum singulare* (Trematoda). Host material came from Canada, Mexico, and the United States.

63. *The possibility of Chemical Control of the Snail Intermediate Hosts of Schistosoma mansoni in Venezuela*. II. GEORGE W. LUTTERMOSER, Instituto Nacional de Higiene, Caracas, Venezuela.

In the laboratory, it was found that a 1:1,000 solution of recently slaked lime (about 50 per cent pure) not only killed the snail intermediate hosts of *Schistosoma*

mansoni but also killed the eggs of the same snails. These eggs failed to develop after remaining in said solution for 6 to 24 hours. A study of the effect of each of the principal components of the lime solution on the snails indicated that most likely the hydroxide present was the factor lethal for the snails. The cercariae of *S. mansoni* died within 15 to 30 minutes in the 1:1,000 lime solution.

This year the following snail-infested zones in populated areas about Caracas were successfully treated with lime: 1500 feet of an irrigation canal with an intermittent flow of water; a 300-foot section of an irrigation canal with continuously running water; a large reservoir; and a section of a river 1500 feet long and 6 to 12 feet wide. Examination of snails collected before and after the first application of the lime indicated that more than 90 per cent of the numerous snails found alive were killed by the treatment. By means of applications of lime every two or three months, it was possible to maintain the first three zones practically free of snails for a test period of six months and at a low expenditure. From the collections made, it was estimated that some 150,000 potential snail carriers of *S. mansoni* were killed in the river and 14 per cent of the collections were found harboring the cercariae of *S. mansoni*.

64. *A Peculiar Larval Development of Rhabdochona spp. (Nematoda: Spiruridae)*. PAUL V. GUSTAFSON, Whitworth College.

The larval development of several species of *Rhabdochona* occurs in various mayfly nymphs. Generic characteristics are established by the end of the second stage, which is followed immediately by encystment. Growth and maturation do not cease but may continue through at least one more molt. In one experiment, a *Hexagenia* nymph seven months after infection contained three excysted males, all of which were apparently mature although they were still partly encased in a shed cuticle. The pattern of the papillae, spicules, head structures, and total body size all agreed with the adult description. Natural infections of *Rhabdochona cascadii* in *Hexagenia* could be identified from the precocious larvae. In several females, unfertilized ova could be seen within the uterus, while many males exhibited mature spicules, papillae pattern, and sperms within the reproductive system. Such precocious development has not been previously reported for this group.

65. *African Sleeping Sickness*. EUGENE R. KELLERSBERGER, American Mission to Lepers, New York.

No abstract received.

AMERICAN SOCIETY OF PARASITOLOGISTS

COUNCIL

Officers for 1942

HENRY E. MELENEY, New York University	<i>President</i>
RUDOLPH W. GLASER, Rockefeller Institute for Medical Research ...	<i>Vice-President</i>
OLIVER R. MCCOY, University of Rochester, and (after May 15)	
JAMES T. CULBERTSON, Columbia University	<i>Secretary</i>
LLOYD E. ROZEBOOM, Johns Hopkins University	<i>Treasurer</i>

Council Member Ex Officio

NORMAN R. STOLL, Rockefeller Institute for Medical Research	<i>Chairman, Editorial Committee</i>
-------------------------------------------------------------------	--------------------------------------

Council Members at Large (with date of expiration of term)

1945	RAYMOND M. CABLE, Purdue University.
1945	WILLARD H. WRIGHT, United States Public Health Service.
1944	DONALD L. AUGUSTINE, Harvard University.
1944	GILBERT F. OTTO, Johns Hopkins University.
1943	CLAY G. HUFF, University of Chicago.
1943	HORACE W. STUNKARD, New York University.
1942	ELERY R. BECKER, Iowa State College.
1942	EMMETT W. PRICE, United States Department of Agriculture.

Representatives of the Society on the Council of the American Association for the Advancement of Science

ASA C. CHANDLER

HARLEY J. VAN CLEAVE

Representatives of the Society on the Council of the Union of American Biological Societies

GEORGE L. GRAHAM

ARTHUR C. WALTON

Editorial Committee of the JOURNAL OF PARASITOLOGY

NORMAN R. STOLL, Chairman	to serve until 1943
WILLIAM H. TALIAFERRO	" " " "
WILLIAM A. RILEY	" " " "

Editorial Board of the JOURNAL OF PARASITOLOGY

WILLIAM W. CORT	to serve until 1945
HAROLD KIRBY, JR.	" " " "
BENJAMIN SCHWARTZ	" " " "
RICHARD P. HALL	" " " 1944
E. HAROLD HINMAN	" " " "
JUSTUS F. MUELLER	" " " "
HAROLD W. BROWN	" " " 1943
HAROLD W. MANTER	" " " "
REGINALD D. MANWELL	" " " "
ASA C. CHANDLER	" " " 1942
CORNELIUS B. PHILIP	" " " "
ERNEST E. TYZZER	" " " "

LIST OF FORMER OFFICERS

President

1925	HENRY B. WARD
1926	CHARLES W. STILES*
1927	RICHARD P. STRONG
1928	CHARLES A. KOFOID
1929	NATHAN A. COBB*
1930	WILLIAM W. CORT
1931	WILLIAM A. RILEY
1932	MAURICE C. HALL*
1933	WILLIAM H. TALIAFERRO
1934	ERNEST E. TYZZER
1935	CHARLES F. CRAIG
1936	ROBERT HEGNER*
1937	GEORGE R. LARUE
1938	FRED C. BISHOPP
1939	HORACE W. STUNKARD
1940	DAVID H. WENRICH
1941	JAMES E. ACKERT

Vice-President

SAMUEL T. DARLING*
CHARLES A. KOFOID
EDWIN LINTON*
ROBERT HEGNER*
GEORGE R. LARUE
ERNEST CARROLL FAUST
ASA C. CHANDLER
WILLIAM H. TALIAFERRO
FRED C. BISHOPP
JAMES E. ACKERT
HARLEY J. VAN CLEAVE
WILLIAM B. HERMS
DAVID H. WENRICH
ELERY R. BECKER
HENRY E. MELENEY
GOTTHOLD STEINER
JUSTIN ANDREWS

Secretary-Treasurer

WILLIAM W. CORT	1925; 1926; 1927; 1928; 1929
NORMAN R. STOLL	1930; 1931; 1932

Secretary

HORACE W. STUNKARD	1933-34; 1935-36; 1937
OLIVER R. MCCOY	1938-39; 1940-41; 1942

Treasurer

JUSTIN ANDREWS	1933-34; 1935-36
GILBERT F. OTTO	1937-38; 1939-40

Council Members at Large

PAUL BARTSCH	1925-28	W. B. HERMS	1930-33
FRED C. BISHOPP	1925-28; 1929-30	BENJAMIN SCHWARTZ	1930-33
ROBERT HEGNER*	1925-27	L. R. CLEVELAND	1931
CHARLES A. KOFOID	1925	W. W. CORT	1931-34; 1935-38
B. H. RANSOM*	1925	H. E. EWING	1931-32
WILLIAM A. RILEY	1925-26; 1928-30	ERNEST C. FAUST	1931-34; 1938-41
CHARLES W. STILES*	1925; 1929-32	JOHN F. KESSEL	1932-35
ERNEST E. TYZZER	1925-26	D. H. WENRICH	1932-35; 1936
MAURICE C. HALL*	1926-29	H. E. MELENEY	1933-36
WILSON G. SMILLIE	1926-27	NORMAN R. STOLL	1933-36; 1937-40;
HENRY B. WARD	1926-29	ELOISE B. CRAM	1934-37 1941
FRANKLIN D. BARKER*	1927-30	WILBUR A. SAWYER	1934-37
J. H. ST. JOHN	1927-28	JAMES E. ACKERT	1935-38
W. H. TALIAFERRO	1928-31	EARL C. O'ROKE	1936-39
ASA C. CHANDLER	1929-30; 1936-39	JUSTIN ANDREWS	1937-40
	HARLEY J. VAN CLEAVE		1938-41

* Deceased.

SOCIETY OFFICERS

33

Editorial Committee of the JOURNAL OF PARASITOLOGY

WILLIAM W. CORT, <i>Chairman</i>	1932-37	FRANCIS M. ROOT*	1932-34
ROBERT HEGNER*	1932-34	WILLIAM A. RILEY	1934-37
WILLIAM H. TALIAFERRO	1934-37		

Editorial Board of the JOURNAL OF PARASITOLOGY

CHARLES F. CRAIG	1932-33; 1933-37	DAVID H. WENRICH	1932-36; 1938-41
MAURICE C. HALL*	1932-33	ERNEST C. FAUST	1933-37
HENRY B. WARD	1932-33	BENJAMIN SCHWARTZ	1933-37; 1938-41
ASA C. CHANDLER	1932-34; 1935-38	ELERY R. BECKER	1934-35; 1936-39
CHARLES A. KOFOID	1932-34; 1935-38	ROBERT MATHESON	1935-38
WILLIAM A. RILEY	1932-34	OLIVER R. MCCOY	1936-39
JAMES E. ACKERT	1932-35	HENRY E. EWING	1937-40
RICHARD P. STRONG	1932-35; 1936-39	JOHN F. KESSEL	1937-40
W. H. TALIAFERRO	1932-34	HARLEY J. VAN CLEAVE	1937-40
FRED C. BISHOPP	1932-36	WILLIAM W. CORT	1938-41
GEORGE R. LARUE	1932-36		

* Deceased.

List of Meeting Places

1925 Kansas City	1930 Cleveland	1936 Atlantic City
1926 Philadelphia	1931 New Orleans	1937 Indianapolis
1927 Nashville	1932 Atlantic City	1938 Richmond
1928 New York	1933 Boston	1939 Columbus
1929 Des Moines	1934 Pittsburgh	1940 Philadelphia
	1935 St. Louis	1941 Dallas

IN MEMORIAM

HARVEY P. BARRET
DONALD CAMERON
MATTHEW E. CARROLL, JR.
RALPH K. COLLINS
†JOHN E. GUBERLET

†ROBERT W. HEGNER
†J. LEE KIRBY-SMITH
†MARCUS W. LYON
†CHARLES W. STILES
†WINFIELD C. SWEET

AMERICAN SOCIETY OF PARASITOLOGISTS

LIST OF MEMBERS¹*Honorary Foreign Members*

- BACIGALUPO, JUAN. Facultad de Medicina, Buenos Aires, Argentina.
 BAYLIS, HARRY A. British Museum (Natural History), Cromwell Road, London, S.W. 7, England.
 BRUMPT, ÉMILE. Laboratoire de Parasitologie, Faculté de Médecine, 15, Rue de l'École de Médecine, Paris VI, France.
 FUHRMANN, OTTO. Institut de Zoologie, Université Neuchâtel, Neuchâtel, Switzerland.
 MARTINI, ERIC. Institut für Schiffs-und Tropenkrankheiten, Hamburg, Germany.
 SERGENT, EDMOND. L'Institut Pasteur d'Algerie, Algerie, North Africa.
 SERGENT, ETIENNE. L'Institut Pasteur d'Algerie, Algerie, North Africa.
 SKRJABIN, KONSTANTIN I. Institut d'Helminthologie de l'École Veterinaire, University of Moscow, Russia.
 SWELLENGREBEL, N. H. Institute for Tropical Hygiene, Mauritskade 57, Amsterdam, The Netherlands.
 TRAVASSOS, LAURO PEREIRA. Instituto Oswaldo Cruz, Rio de Janeiro, Brazil.
 WENYON, CHARLES MORLEY. Wellcome Research Institution, 183 Euston Road, London, England.

Active Members

Three life members indicated by*

Charter members indicated by†

- †ACKERT, JAMES E. Department of Zoology, Kansas State College of Agriculture and Applied Science, Manhattan, Kans.
 ACOSTA, JOSEFINA. Biological Laboratory, Department of Public Health, San Juan, Puerto Rico.
 ADAMS, J. ALFRED. Department of Zoology, Grinnell College, Grinnell, Iowa.
 ALICATA, JOSEPH E. University of Hawaii, Honolulu, H. I.
 †ALLEN, ENA A. Zoological Division, U. S. Bureau of Animal Industry, Beltsville Research Center, Beltsville, Md.
 ALLEN, ROBERT M. Department of Pathology and Bacteriology, College of Medicine, University of Nebraska, Omaha, Nebr.
 ALLISON, LEONARD N. State Fish Hatchery, Grayling, Mich.
 AMEEL, DONALD J. Department of Zoology, Kansas State College of Agriculture and Applied Science, Manhattan, Kans.
 ANDERSON, CYRUS V. 221 West 6th Street, Winona, Minn.
 ANDERSON, MARLOWE G. Department of Biology, New Mexico State College, State College, N. Mex.
 †ANDREWS, JUSTIN. Division of Malaria Investigation, State Department of Public Health, Atlanta, Ga.
 ANDREWS, JOHN S. Coastal Plain Experiment Station, U. S. Bureau of Animal Industry, Tifton, Ga.
 ANNEXEAUX, RALPH F. Animal Pathology Laboratory, Department of Agriculture, Sacramento, Calif.
 ARANT, FRANK S. Department of Zoology and Entomology, Alabama Polytechnic Institute, Auburn, Ala.
 ARNOLD, JOHN G., JR. Department of Biology, Loyola University, New Orleans, La.
 †AUGUSTINE, DONALD L. Department of Comparative Pathology, Harvard Medical School, Boston, Mass.

¹ As of November 1, 1942. The last preceding list of members was published in the JOURNAL OF PARASITOLOGY, 1939, 25 Suppl: 41. Earlier lists were in 1926, 12: 170 and 1934, 20: 345.

- AUGUSTSON, G. F. Allan Hancock Foundation, University of Southern California, Los Angeles, Calif.
- AVERY, JOHN L. Zoological Division, U. S. Bureau of Animal Industry, Beltsville Research Center, Beltsville, Md.
- BACHMAN, GEORGE W. Germantown, Montgomery County, Ohio.
- BAKER, DONALD W. Department of Parasitology, New York State Veterinary College, Cornell University, Ithaca, N. Y.
- †BALL, GORDON H. 405 Hilgard Avenue, University of California at Los Angeles, Los Angeles, Calif.
- †BANGHAM, RALPH V. Department of Biology, College of Wooster, Wooster, Ohio.
- BARD, GREGORY. 66 Panoramic Way, Berkeley, Calif.
- BARLOW, CLAUDE H. 102 Crescent Avenue, Leonia, New Jersey.
- BARRETT, JOHN P. Research Laboratory, Armour and Company, Union Stock Yards, Chicago, Ill.
- †BARTSCH, PAUL S. U. S. National Museum, Washington, D. C.
- BASNUEVO ARTILES, JOSE G. Department of Parasitology, University of Havana, Havana, Cuba.
- BASSEN, JONAS L. U. S. Bureau of Entomology and Plant Quarantine, 138 College Street, Buffalo, N. Y.
- †BEAUDETTE, FRED R. New Jersey Agricultural Experiment Station, New Brunswick, N. J.
- BEAVER, PAUL C. State Department of Public Health, Regional Office, Waycross, Ga.
- †BECKER, ELERY R. Department of Zoology, Iowa State College, Ames, Iowa.
- BEERS, CHARLES D. Department of Zoology, University of North Carolina, Chapel Hill, N. C.
- BELKIN, JOHN N. Health and Safety Department, Tennessee Valley Authority, Wilson Dam, Ala.
- BELTRAN, ENRIQUE. Department of Protozoology, Institute of Public Health and Tropical Diseases, Mexico City, Mexico.
- BENARROCH, ELIAS. Division de Malaria, Dirrecion de Salubridad Publica, Ministerio de Sanidad y Asistencia Social, Caracas, Venezuela.
- †BENBROOK, EDWARD A. Division of Veterinary Pathology, Iowa State College, Ames, Iowa.
- BENNETT, HARRY J. Department of Zoology, Louisiana State University, University, La.
- BENNINGTON, ELWIN E. Berkeley, Tex.
- BERBERIAN, DICRAN A. Department of Bacteriology and Parasitology, The American University of Beirut, Beirut, Syria.
- BIDDLE, MARJORIE O. School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Md.
- †BISHOPP, FRED C. Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Washington, D. C.
- BLACK, JAMES J. New Jersey State Poultry Laboratory, Vineland, N. J.
- BOARDMAN, EDWARD T. Cranbrook Institute of Science, Bloomfield Hills, Mich.
- BOND, FRANKLYN F. Department of Bacteriology, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.
- BONESTELL, AILEEN E. Agricultural Experiment Station, University of California, Berkeley, Calif.
- BOUGHTON, DONALD C. Zoological Division, U. S. Bureau of Animal Industry, Beltsville Research Center, Beltsville, Md.
- BOYD, GEORGE H. Department of Zoology, University of Georgia, Athens, Ga.
- †BOYD, MARK F. International Health Division, Rockefeller Foundation, Tallahassee, Fla.
- BRACKETT, STERLING. School of Public Health, University of North Carolina, Chapel Hill, N. C.

- BRADBURY, ORA C. Department of Biology, Wake Forest College, Wake Forest, N. C.
- BRADY, FREDERICK J. Division of Zoology, National Institute of Health, Bethesda, Md.
- BRANCH, HAZEL E. Department of Zoology, University of Wichita, Wichita, Kans.
- BRANDT, BARTHOLOMEW B. Department of Biology, East Carolina Teacher's College, Greenville, N. C.
- BRECKENRIDGE, CARLYLE G. Department of Zoology, University of Alabama, University, Ala.
- BRODY, ARTHUR L. 111 East 2nd Street, Corning, New York.
- BROOKE, MARION M. Department of Parasitology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Md.
- †BROOKS, FRANK G. Department of Biology, Cornell College, Mount Vernon, Iowa.
- †BROWN, HAROLD W. Department of Preventive Medicine, University of North Carolina, Chapel Hill, N. C.
- BROWN, VIRGINIUS E. Department of Biology, Taylor University, Upland, Ind.
- BROWNE, PATRICK. Department of Animal and Plant Pathology, Rockefeller Institute for Medical Research, Princeton, N. J.
- BUNTING, MARTHA. 1520 Spruce Street, Philadelphia, Penn.
- BURGESS, ROBERT W. U. S. Public Health Service, Columbia, S. C.
- BURSTEIN, HAROLD. Bureau of Animal Industry, 4148 Penns Grove Street, Philadelphia, Penn.
- BYRD, ELON E. Department of Zoology, University of Georgia, Athens, Ga.
- CABALLERO Y CABALLERO, EDUARDO. Instituto de Biología, Chapultepec Casa del Lago, Mexico City, Mexico.
- CABLE, RAYMOND M. Department of Biology, Purdue University, West Lafayette, Ind.
- †CAHN, ALVIN R. Biological Readjustment Division, Tennessee Valley Authority, Norris, Tenn.
- †CAMERON, ALNE E. Chief Veterinary Inspector, 231 Sunnyside Avenue, Ottawa, Ontario, Canada.
- CAMERON, THOMAS W. M. Institute of Parasitology, MacDonald College, McGill University, Montreal, Canada.
- CANNING, GRAEME A. Department of Zoology and Parasitology, University of Tennessee, Knoxville, Tenn.
- CANTRELL, WILLIAM F. Department of Bacteriology and Parasitology, University of Chicago, Chicago, Ill.
- †CARROLL, MITCHELL. Department of Biology, Franklin and Marshall College, Lancaster, Penn.
- CARTER, THOMAS C. Department of Biology, Northwestern State Teachers College, Alva, Okla.
- CARVALHO, JOSE C. M. Department of Zoology and Entomology, Iowa State College, Ames, Iowa.
- CASE, ARTHUR A. Zoology Department, Kansas State College of Agriculture and Applied Science, Manhattan, Kans.
- CAUSEY, OTTIS R. Servico de Malaria do Nordeste, Fortaleza, Brazil.
- †CHANDLER, ASA C. Department of Biology, Rice Institute, Houston, Tex.
- CHANDLER, WALLACE L. Department of Parasitology, Michigan State College, East Lansing, Mich.
- CHANG, KWEL. Department of Biology, Cheeloo University, Chengtu, Szechwan, China.
- CHANG SHIH, L. Department of Comparative Pathology, Harvard Medical School, Boston, Mass.
- CHEN, PIN-DJI. Ginling College, West China University, Chengtu, Szechwan, China.
- CHITWOOD, BENJAMIN G. Bureau of Plant Industry, U. S. Department of Agriculture, Babylon, N. Y.

- CHITWOOD, MAY BELLE. Bureau of Plant Industry, U. S. Department of Agriculture, Babylon, N. Y.
- CHRISTENSEN, JOHN F. Zoological Division, U. S. Bureau of Animal Industry, Beltsville Research Center, Beltsville, Md.
- CHRISTENSON, REED O. Department of Zoology and Entomology, Alabama Polytechnic Institute, Auburn, Ala.
- †CHRISTIE, JESSIE R. Division of Nematology, U. S. Bureau of Plant Industry, Washington, D. C.
- CHURCHILL, HELEN. Department of Zoology, University of Michigan, Ann Arbor, Mich.
- †CLARK, HERBERT C. Gorgas Memorial Laboratory, Apartado 1252, Panama, R. P.
- CLARK, ROBERT D. Research Department, Ralston-Purina Company, St. Louis, Mo.
- †CLEVELAND, L. R. Department of Zoology, Harvard University, Cambridge, Mass.
- COATNEY, G. ROBERT. Division of Chemotherapy, National Institute of Health, Washington, D. C.
- COGGESHALL, LOWELL T. Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, Mich.
- CONNELL, FRANK H. Department of Zoology, Dartmouth College, Hanover, N. H.
- †CORT, WILLIAM W. Department of Parasitology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Md.
- COULSTON, FREDERICK. Department of Bacteriology and Parasitology, University of Chicago, Chicago, Ill.
- †COVENTRY, FRANCES A. 158 Wilson Drive, Lancaster, Penn.
- *†CRAIG, CHARLES F. 239 W. Lullwood Avenue, San Antonio, Tex.
- †CRAM, ELOISE B. Stream Pollution Laboratories, East 3rd and Kilgour Streets, Cincinnati, Ohio.
- CRAWFORD, WILEY W. Department of Biology, Blackburn College, Carlinville, Ill.
- CROSS, SAMUEL X. 908 U. S. Custom House, Chicago, Ill.
- CUCKLER, ASHTON C. Department of Zoology, University of Minnesota, Minneapolis, Minn.
- CULBERTSON, JAMES T. Department of Bacteriology, College of Physicians and Surgeons, Columbia University, New York, N. Y.
- DANIEL, GEORGE E. Department of Parasitology, Live Stock Sanitary Service, University of Maryland, College Park, Md.
- D'ANTONI, JOSEPH S. Department of Tropical Medicine, Tulane University, New Orleans, La.
- †DAVIS, HERBERT S. U. S. Bureau of Fisheries, Washington, D. C.
- DAVIS, REID. Regional Laboratory, U. S. Bureau of Animal Industry, Auburn, Ala.
- DEGIUSTI, DOMINIC L. Department of Veterinary Science, University of Wisconsin, Madison, Wis.
- DE JESUS, ZACARIAS. Division of Parasitology, Bureau of Animal Industry, Manila, P. I.
- †DE RIVAS, DÁMASO. Department of Parasitology, University of Pennsylvania Medical School, Philadelphia, Penn.
- DE TURK, WILLIAM E. Department of Biology, Vanderbilt University, Nashville, Tenn.
- DEHNBOSTEL, NELLIE G. 178 Iddings Avenue N.E., Warren, Ohio.
- DELAUNE, ELAINE T. Veterinary Science Department, Louisiana State Experiment Station, University, La.
- DENNIS, E. WESTERVELT. Department of Bacteriology and Parasitology, The American University of Beirut, Beirut, Syria.
- DENTON, J. FRED. Department of Bacteriology and Public Health, Georgia Medical School, Augusta, Ga.
- DERBYSHIRE, RUSSELL C. Department of Zoology, Municipal University of Omaha, Omaha, Nebr.
- DICKERMAN, E. EUGENE. Department of Biology, Bowling Green State University, Bowling Green, Ohio.

- DOBROVOLNY, CHARLES G. Department of Zoology, University of New Hampshire, Durham, N. H.
- DOETSCHMAN, WILLIS H. Biological Research Institute, San Diego Zoological Society, San Diego, Calif.
- DONALDSON, ALAN W. Department of Parasitology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Md.
- DOUGHERTY, ELLSWORTH C. Department of Zoology, University of California, Berkeley, Calif.
- †DOVE, WALTER E. Box 789, Panama City, Fla.
- DOWNS, WILBUR G. International Health Division, Rockefeller Foundation, Port of Spain, Trinidad, British West Indies.
- †DRAKE, CARL J. Department of Zoology and Entomology, Iowa State College, Ames, Iowa.
- EARLE, HILTON H., JR. 712 East Carocas Street, Tampa, Fla.
- EDGAR, S. ALLEN. Zoology Department, Kansas State College of Agriculture and Applied Science, Manhattan, Kans.
- EISENBRANDT, LESLIE L. Department of Biology, University of Kansas City, Kansas City, Mo.
- ELISHEWITZ, HAROLD. Department of Zoology, University of Minnesota, Minneapolis, Minn.
- EMERSON, KARY C. Fort William McKinley, Rizal, Philippine Islands.
- †ENDERS, HOWARD E. Department of Biology, Purdue University, West Lafayette, Ind.
- ENZIE, FRANK D. Zoological Division, U. S. Bureau of Animal Industry, Beltsville Research Center, Beltsville, Md.
- ERICKSON, ARNOLD B. Division of Economic Zoology, University of Minnesota, St. Paul, Minn.
- †ESSEX, HIRAM E. Department of Physiology, Mayo Foundation, University of Minnesota, Rochester, Minn.
- †EWING, HENRY E. U. S. National Museum, Washington, D. C.
- FALLIS, A. MURRAY. Ontario Research Foundation, 43 Queen's Park, Toronto, Ontario, Canada.
- FARR, MARION M. Zoological Division, U. S. Bureau of Animal Industry, Beltsville Research Center, Beltsville, Md.
- †FAUST, ERNEST C. Department of Tropical Medicine, Tulane University Medical School, New Orleans, La.
- FELDMAN, SEYMOUR I. Department of Zoology, University of Minnesota, Minneapolis, Minn.
- FERGUSON, MALCOLM S. Department of Animal and Plant Pathology, Rockefeller Institute for Medical Research, Princeton, N. J.
- FISCHTHAL, JACOB H. Department of Zoology, University of Michigan, Ann Arbor, Mich.
- FISH, FREDERICK F. U. S. Bureau of Fisheries, Seattle, Wash.
- FISHER, ANNA M. Grant Hospital School of Nursing, 536 Webster Avenue, Chicago, Ill.
- FLORENCE, LAURA. Department of Bacteriology, New York Medical College, New York, N. Y.
- FOSTER, AUREL O. Zoological Division, U. S. Bureau of Animal Industry, Beltsville Research Center, Beltsville, Md.
- FRENCH, WILLIAM G. St. Mary's Hospital and French Hospital, Evansville, Ind.
- FRENKEL, JACOB. Department of Zoology, University of California, Berkeley, Calif.
- FRICK, LYMAN P. Zoology Department, Kansas State College of Agriculture and Applied Science, Manhattan, Kans.
- FURMAN, DEANNE P. Medical Detachment, Station Hospital, Fort Ord, Calif.
- GALLO, PIERRO. Departamento de Enfermedades Infecciosas y Parasitarias en la Escuela Superior de Medicina Veterinaria de Caracas, Caracas, Venezuela.

- GABALDON, ARNALDO. Ministerio de Sanidad y Asistencia Social, Caracas, Venezuela.
- GAN, TOMAS M. Institute of Hygiene, University of the Philippines, Manila, P. I.
- GARCIA-DIAZ, JULIO. Department of Biology, University of Puerto Rico, Rio Piedras, P. R.
- GEIMAN, QUENTIN M. Department of Comparative Pathology, Harvard Medical School, Boston, Mass.
- GINGRICH, WENDELL. Department of Bacteriology and Preventive Medicine, University of Texas Medical School, Galveston, Tex.
- GLASER, RUDOLPH W. Department of Animal and Plant Pathology, Rockefeller Institute for Medical Research, Princeton, N. J.
- GLASSMAN, JEROME M. 5320 First Street, N.W., Washington, D. C.
- GOBLE, FRANS C. Game Research Center, Delmar, New York.
- GOLDHAFT, TEUIS M. Vineland Poultry Laboratory, Vineland, N. J.
- GOLDMAN, MORRIS. U. S. Public Health Service, San Antonio, Tex.
- GONZALEZ-MARTINEZ, ISAAC F. Roentgoenological Clinic, Diaz Garcia Hospital, San Juan, Puerto Rico.
- GOODCHILD, CHAUNCEY G. Department of Biology, State Teachers College, Springfield, Mo.
- GOODNIGHT, CLARENCE J. 345 Natural History Building, University of Illinois, Urbana, Ill.
- GOWER, CARL. 224 Bailey Street, East Lansing, Mich.
- GRAHAM, GEORGE L. Department of Animal and Plant Pathology, Rockefeller Institute for Medical Research, Princeton, N. J.
- †GRAHAM, JOHN Y. Department of Biology, University of Alabama, University, Ala.
- GREEN, NANCY K. V. Mississippi State Hygienic Laboratory, Jackson, Miss.
- GRIFFITHS, HENRY J. Department of Veterinary Pathology, Iowa State College, Ames, Iowa.
- †GRILL, JOHN C. Department of Pathology and Bacteriology, School of Medicine, Marquette University, Milwaukee, Wis.
- GURSCH, OTTO F. Station Hospital, Camp Sutton, N. C.
- GUSTAFSON, PAUL V. Department of Zoology, Whitworth College, Spokane, Wash.
- †HADWEN, SEYMOUR. Department of Pathology and Bacteriology, Ontario Research Foundation, 47 Queen's Park, Toronto, Ontario, Canada.
- †HALL, RICHARD P. Department of Biology, New York University, University Heights, New York, N. Y.
- HAMANN, CECIL B. Department of Bacteriology, University of Kentucky, Lexington, Ky.
- †HANNUM, CLAIR A. 1014 W. Blaine Street, Seattle, Wash.
- HANSEN, MERLE F. 1303 East 28th Street, Minneapolis, Minn.
- HANSON, HENRY. Traveling Representative, Pan American Sanitary Bureau, Washington, D. C.
- HARDCASTLE, A. BASCOM. Zoological Division, U. S. Bureau of Animal Industry, Beltsville Research Center, Beltsville, Md.
- HARKEMA, REINARD. Department of Zoology, North Carolina State College, Raleigh, N. C.
- HART, THOMAS A. Station Hospital, Camp Gordon, Ga.
- †HARTMAN, ERNEST. Department of Bacteriology and Clinical Pathology, University of Vermont College of Medicine, Burlington, Vt.
- HARWOOD, PAUL D. 203 Ferrell Avenue, Ashland, Ohio.
- †HATHAWAY, EDWARD S. Department of Zoology, Tulane University, New Orleans, La.
- HAUSCHKA, THEODORE S. Department of Zoology, University of Pennsylvania, Philadelphia, Penn.
- HAWKINS, PHILIP A. Department of Bacteriology, Michigan State College, East Lansing, Mich.

- HAZARD, FRANK O. Department of Zoology, Wilmington College, Wilmington, Ohio.
- HEADLEE, WILLIAM H. Department of Biology, Purdue University, West Lafayette, Ind.
- HEARIN, JAMES T. Department of Preventive Medicine and Public Health, School of Medicine, University of Texas, Galveston, Tex.
- HEDRICK, LESLIE R. Biology Department, Illinois Institute of Technology, Chicago, Ill.
- HERBER, ELMER C. Department of Biology, Dickinson College, Carlisle, Penn.
- HERMAN, CARLTON M. California Division of Fish and Game, Ferry Building, San Francisco, Calif.
- †HERMS, WILLIAM B. Agricultural Hall, University of California, Berkeley, Calif.
- †HERRICK, CHESTER A. Department of Zoology, University of Wisconsin, Madison, Wis.
- HERTIG, MARSHALL. Care of American Consulate, Lima, Peru.
- †HETHERINGTON, DUNCAN C. Department of Anatomy, Duke University, Durham, N. C.
- HEWITT, REDGINAL I. Laboratory Services Division, Tennessee Valley Authority, Wilson Dam, Ala.
- HIGHBY, PAUL R. Department of Zoology, University of Minnesota, Minneapolis, Minn.
- HILL, CHARLES H. Zoological Division, U. S. Bureau of Animal Industry, Beltsville Research Center, Beltsville, Md.
- HILL, HOWARD R. Los Angeles Museum Exposition Park, Los Angeles, Calif.
- HILL, ROLLA B. International Health Division, Rockefeller Foundation, Havana, Cuba.
- HINMAN, E. HAROLD. Health Section, Tennessee Valley Authority, Wilson Dam, Ala.
- HIYEDA, KENTARO. Department of Pathology and Parasitology, Manchuria Medical College, Mukden, Manchoukuo.
- HOBMAIER, MICHAEL. Department of Comparative Pathology, Hooper Foundation for Medical Research, University of California, San Francisco, Calif.
- †HOEPLI, REINHARD J. Department of Parasitology, Peiping Union Medical College, Peiping, China.
- †HOFFMAN, WILLIAM A. Department of Parasitology, School of Tropical Medicine, San Juan, Puerto Rico.
- HOGUE, MARY J. Department of Anatomy, University of Pennsylvania Medical School, Philadelphia, Penn.
- †HOLL, FREDERICK J. Biology Department, University of Buffalo, Buffalo, N. Y.
- HOPKINS, SEWELL H. Department of Biology, Texas Agricultural and Mechanical College, College Station, Tex.
- HOPP, WILLIAM B. Department of Biology, Purdue University, Lafayette, Ind.
- HORSFALL, MARGERY W. Route 2, Fairport, New York.
- HOUGHTON, HENRY S. Peiping Union Medical College, Peiping, China.
- HSIUNG, T. S. Szechuan Provincial Bureau of Animal Industry, South Gate, Chengtu, Szechuan, China.
- HSU, DORIS YIN-MING. Hwa Nan College, Foochow, China.
- HUDSON, CHARLES B. New Jersey Agricultural Experiment Station, New Brunswick, N. J.
- †HUFF, CLAY G. Department of Bacteriology and Parasitology, University of Chicago, Chicago, Ill.
- HUFF, GEORGE C. Department of Biology, Drake University, Des Moines, Iowa.
- HUGHES, R. CHESTER. Department of Zoology, Oklahoma Agricultural and Mechanical College, Stillwater, Okla.
- HUMES, ARTHUR G. R.F.D., No. 4, Attleboro, Mass.
- HUNNINEN, ARNE V. Department of Biology, Oklahoma City University, Oklahoma City, Okla.

- [†]HUNTER, GEORGE W., III. Army Medical School, Army Medical Center, Washington, D. C.
- HUNTER, WANDA S. 10980 Rochester Avenue, Village Station, Los Angeles, Calif.
- HURLBUT, HERBERT S. Marine Barracks, Roosevelt Roads, Vieques Island, Puerto Rico.
- HUSSEY, KATHLEEN L. Department of Biology, Meredith College, Raleigh, N. C.
- IRIARTE, DAVID R. Universidad Central, Clinica Razetti, Los Caobos, Caracas, Venezuela.
- ISHII, NOBUTARO. Institute of Infectious Diseases, Tokyo Imperial University, Tokyo, Japan.
- JACOBS, LEON. Division of Zoology, National Institute of Health, Bethesda, Md.
- JACOBS, WILLIAM F. Department of Pathology, Buffalo City Hospital, Buffalo, N. Y.
- JAMES, WILLIAM M. The Herrick Clinic, 12 Central Avenue, Panama, R. P.
- JANKIEWICZ, HARRY A. 4258 South Main Street, Los Angeles, Calif.
- JELLISON, WILLIAM L. Rocky Mountain Laboratory, U. S. Public Health Service, Hamilton, Mont.
- JERSTAD, ARTHUR C. 4535 Lindell Boulevard, St. Louis, Mo.
- [†]JOHNSON, JOHN C. State Teachers College, Edinboro, Penn.
- JOHNSTONE, HERBERT G. Department of Bacteriology, University of California Medical School, Medical Center, San Francisco, Calif.
- JONES, ARTHUR W. Miller School of Biology, University of Virginia, University, Va.
- JONES, FRANCES. Division of Zoology, National Institute of Health, Bethesda, Md.
- JONES, MYRNA F. Division of Zoology, National Institute of Health, Bethesda, Md.
- JORDAN, HELEN B. Department of Bacteriology and Parasitology, University of Chicago, Chicago, Ill.
- KADNER, CARL G. Department of Biology, Loyola University of Los Angeles, Los Angeles, Calif.
- KATES, KENNETH C. Zoological Division, U. S. Bureau of Animal Industry, Beltsville Research Center, Beltsville, Md.
- KEANE, ROGER H. U. S. Naval Hospital, Oakland, Calif.
- KELLEY, RUTH SHAW. 321 East Erie Street, Kent, Ohio.
- KERR, JOHN A. International Health Division, Rockefeller Foundation, Caixa Postal 49, Rio de Janeiro, Brazil.
- KERR, KATHEL B. Division of Zoology, National Institute of Health, Bethesda, Md.
- [†]KESSEL, JOHN F. Department of Bacteriology and Parasitology, School of Medicine, University of Southern California, Los Angeles, Calif.
- KESSLER, WALTER. Department of Bacteriology, Columbia University, New York, N. Y.
- KHAW, OO-KEK. Department of Parasitology, Peiping Union Medical College, Peiping, China.
- KILLEEN, JOHN A. DePauw University, Greencastle, Ind.
- [†]KING, WILLARD V. Fourth Corps Area Laboratory, Fort McPherson, Ga.
- KIRBY, HAROLD, JR. Department of Zoology, University of California, Berkeley, Calif.
- *[†]KOFOID, CHARLES A. Department of Zoology, University of California, Berkeley, Calif.
- KOHLRUSS, FRED J. Department of Biology, University of Portland, Portland, Ore.
- KOHL, GLEN M. U. S. Public Health Service, Hamilton, Mont.
- KOONZ, CARL H. Chemical Laboratory, Swift and Company, Chicago, Ill.
- KOTCHER, EMIL. State Department of Health, Jackson, Miss.
- KOURI-ESMEJA, PEDRO. Laboratorios Kuba, Concordia No. 65, Havana, Cuba.
- KOWALCZYK, STANLEY A. 825 Newmarket Street, Philadelphia, Penn.
- KRUIDENIER, FRANCIS. 917 Edgewood Place, Ann Arbor, Mich.
- [†]KUDO, RICHARD R. Department of Zoology, University of Illinois, Urbana, Ill.

- KUNTZ, ROBERT E. Department of Zoology, University of Michigan, Ann Arbor, Mich.
- †LAAKE, ERNEST W. U. S. Bureau of Entomology and Plant Quarantine, Dallas, Tex.
- LAIRD, RAYMOND L. School of Public Health, University of Michigan, Ann Arbor, Mich.
- LANDSBERG, J. W. Warner Institute for Therapeutic Research, 113 West 18th Street, New York, N. Y.
- †LANGE, MATHILDE M. Department of Zoology, Wheaton College, Norton, Mass.
- LARSH, JOHN E., JR. Department of Parasitology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Md.
- †LARSON, MARY E. Department of Zoology, University of Kansas, Lawrence, Kans.
- †LARUE, GEORGE R. Department of Zoology, University of Michigan, Ann Arbor, Mich.
- LAUZUN, VIRGINIA. Game Division, Michigan State Department of Conservation, Lansing, Mich.
- LAWLER, HARRY J. Department of Bacteriology, School of Medicine, Washington University, St. Louis, Mo.
- LAWLOR, WILLIAM K. U. S. Naval Air Station, Trinidad, British West Indies.
- LEROUX, PHILIPPUS L. Veterinary Research Station, Marabuka, North Rhodesia, South Africa.
- LEADINGHAM, ROY S. Department of Medicine, Emory University, Atlanta, Ga.
- LEIGH, W. HENRY. 920 Hastings Avenue, Park Ridge, Ill.
- LENT, HERMAN. Instituto Oswaldo Cruz., Rio de Janeiro, Brazil.
- LEONARD, A. B. Department of Zoology, University of Kansas, Lawrence, Kans.
- LEONARD, ALICE S. Department of Zoology, University of Kansas, Lawrence, Kans.
- LEVIN, ARTHUR J. Department of Zoology, University of Iowa, Iowa City, Iowa.
- LEVINE, PINCUS P. New York State Veterinary College, Cornell University, Ithaca, N. Y.
- LINCICOME, DAVID R. Department of Zoology, University of Kentucky, Lexington, Ky.
- LOEWEN, SOLOMON L. Tabor College, Hillsboro, Kans.
- LOWELL, RALPH D. Department of Zoology, University of Illinois, Urbana, Ill.
- LUCKER, JOHN T. Zoological Division, U. S. Bureau of Animal Industry, Beltsville Research Center, Beltsville, Md.
- LUDLOW, ALFRED I. Department of Surgery, Severance Union Medical College, Seoul, Korea.
- LUDWIG, FRANCIS W. Villanova College, Villanova, Penn.
- LUND, EVERETT E. Department of Biology, Alfred University, Alfred, N. Y.
- LUNDAHL, WALTER S. Department of Zoology, University of Michigan, Ann Arbor, Mich.
- LUTTERMOSER, GEORGE W. 5558 Ivanhoe Avenue, Detroit, Mich.
- †LYNCH, JAMES E. School of Fisheries, University of Washington, Seattle, Wash.
- †LYNCH, KENNETH M. Department of Pathology, Medical College of the State of South Carolina, Charleston, S. C.
- MACLULICH, DUNCAN A. 144 Mavety Street, Toronto, Canada.
- MACY, RALPH W. Department of Biology, Reed College, Portland, Ore.
- †MAGATH, THOMAS B. Department of Pathology and Parasitology, Mayo Foundation, University of Minnesota, Rochester, Minn.
- MALDONADO, JOSE F. School of Tropical Medicine, San Juan, Puerto Rico.
- MANGRUM, JAMES F. Zoology Department, Duke University, Durham, N. C.
- †MANter, HAROLD W. Department of Zoology, University of Nebraska, Lincoln, Nebr.
- MANWELL, REGINALD D. Department of Zoology, Syracuse University, Syracuse, N. Y.
- MARIN, RAFAEL A. 15 West 110th Street, New York, N. Y.

- †MARK, EDWARD L. Department of Anatomy, Harvard University, Cambridge, Mass.
- MARKELL, EDWARD K. 1120 Cragmont Avenue, Berkeley, Calif.
- MARQUES ESCOBEDO, MANUEL B. Mexican Department of Health, Edificio Condesa-M-4, Mexico City, Mexico.
- MARTIN, HARVEY A. Department of Zoology, University of Nebraska, Lincoln, Nebr.
- †MARTIN, H. M. Department of Veterinary Pathology and Parasitology, University of Pennsylvania, Philadelphia, Penn.
- MARTIN, WALTER E. Department of Zoology, DePauw University, Greencastle, Ind.
- MARTINEZ-BAEZ, MANUEL. Department of Pathology, Institute of Public Health and Tropical Diseases, Mexico City, Mexico.
- †MATHESON, ROBERT. Department of Entomology, Cornell University, Ithaca, N. Y.
- MAUSS, EVELYN A. Hot Springs, S. Dak.
- MAYFIELD, ORLEY J. Charles City, Iowa.
- †MAYHEW, ROY L. Department of Zoology, Louisiana State University, University, La.
- MAZZOTTI, LUIS. Laboratory of Helminthology, Institute of Tropical Diseases, Mexico City, Mexico.
- MCCAFFERY, JOSEPH C. Springfield Junior College, Springfield, Ill.
- MCCOY, OLIVER R. Department of Bacteriology, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.
- †McCULLOCH, IRENE A. Department of Zoology, Allan Hancock Foundation, University of Southern California, Los Angeles, Calif.
- McDERMOTT, PETER H. Florida State Board of Health, Tallahassee, Fla.
- McFARLANE, SAMUEL H. Department of Biology, Aurora College, Aurora, Ill.
- McHUGH, ROBERT A. Department of Parasitology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Md.
- †McINTOSH, ALLEN. Zoological Division, U. S. Bureau of Animal Industry, Beltsville Research Center, Beltsville, Md.
- McIVOR, BARBARA C. Division of Preventive Medicine, University of California Medical School, San Francisco, Calif.
- McMULLEN, DONALD B. Bacteriology Department, University of Oklahoma Medical School, Oklahoma City, Okla.
- McNAUGHT, JAMES B. Department of Pathology, School of Medicine, Stanford University, San Francisco, Calif.
- McNEIL, CHARLES W. Department of Zoology, University of Michigan, Ann Arbor, Mich.
- McNEIL, ETHEL C. E. Department of Veterinary Science, University of California, Davis, Calif.
- †MELENEY, HENRY E. Department of Preventive Medicine, College of Medicine, New York University, New York, N. Y.
- †MESERVE, FRANK G. Department of Biology, Macalester College, St. Paul, Minn.
- MEYER, MARVIN C. Zoology Department, New Jersey State College for Women, New Brunswick, N. J.
- MICKLER, ROBERT H. Department of Preventive Medicine, University of Tennessee, Memphis, Tenn.
- †MIDDLETON, AUSTIN R. Department of Biology, University of Louisville, Louisville, Ky.
- MILES, VIRGIL I. Division of Zoology, National Institute of Health, Bethesda, Md.
- MILLER, EDWIN L. Department of Biology, Lawrence College, Appleton, Wis.
- †MILLER, HARRY M., JR. Rockefeller Foundation, 49 West 49th Street, New York, N. Y.
- MILLER, MAX J. Institute of Parasitology, MacDonald College, McGill University, Quebec, Canada.

- MIZELLE, JOHN D. Department of Biology, University of Notre Dame, Notre Dame, Ind.
- MOHR, JOHN L. Department of Zoology, University of California, Berkeley, Calif.
- MÖNNIG, HERMANN O. Department of Agriculture, Union of South Africa, Onderstepoort, Transvaal, South Africa.
- MOORE, DONALD V. State Board of Health, Bureau of Laboratories, Austin, Tex.
- †MOORE, J. PERCY. Zoological Laboratories, University of Pennsylvania, Philadelphia, Penn.
- MOREHOUSE, NEAL F. 205 4th Street, Charles City, Iowa.
- MORELAND, GEORGE E. Houghton, New York.
- MORGAN, BANNER BILL. Department of Veterinary Science, University of Wisconsin, Madison, Wis.
- MORRIS, SAMUEL. District No. 4, U. S. Public Health Service, New Orleans, La.
- MOSS, EMMA S. Department of Pathology, Charity Hospital, New Orleans, La.
- MOST, HARRY. Department of Clinical Pathology, New York University School of Medicine, New York, N. Y.
- †MUELLER, JUSTUS F. Department of Forest Zoology, New York State College of Forestry, Syracuse, N. Y.
- †MUGRAGE, EDWARD R. Department of Clinical Pathology, University of Colorado Medical School, Denver, Colo.
- MUKERJI, ARUN K. Department of Helminthological Research, Calcutta School of Tropical Medicine, Calcutta, India.
- MUMFORD, EDWARD P. Department of Biology, Stanford University, Palo Alto, Calif.
- NAIMAN, DOROTHY N. Department of Physiology and Health, Hunter College, New York, N. Y.
- NELSON, E. CLIFFORD. State of Maine, Department of Inland Fisheries and Game, Dry Mills, Maine.
- †NELSON, THURLOW C. Department of Zoology, Rutgers University, New Brunswick, N. J.
- NICKEL, HANNAH S. Mississippi State Board of Health, Jackson, Miss.
- NICHOLS, THEODORE E. U. S. Bureau of Animal Industry, Columbus, Ohio.
- NIGRELLI, ROSS F. Aquarium, New York Zoological Park, Bronx, N. Y.
- NOBLE, ALDEN E. P. O. Box 443, Stockton, Calif.
- NOBLE, ELMER R. Department of Biology, State College, Santa Barbara, Calif.
- †NOLAN, MRS. MABELLE O. Division of Zoology, National Institute of Health, Bethesda, Md.
- †NOLAND, LOWELL E. Department of Zoology, University of Wisconsin, Madison, Wis.
- NOLF, LUTHER O. Department of Zoology, University of Iowa, Iowa City, Iowa.
- OFFUTT, EDWARD P., JR. Department of Bacteriology, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.
- OLIVER-GONZALEZ, JOSE. Department of Bacteriology and Parasitology, University of Chicago, Chicago, Ill.
- OLIVIER, LOUIS, JR. Zoological Division, U. S. Bureau of Animal Industry, Beltsville Research Center, Beltsville, Md.
- OLSEN, O. WILFORD. Zoological Division, U. S. Bureau of Animal Industry, Texas Experiment Station, Angleton, Tex.
- OOSTING, MELVIN. Miami Valley Hospital, Dayton, Ohio.
- †O'ROKE, EARL C. Department of Forest Zoology, University of Michigan, Ann Arbor, Mich.
- †OSBORN, HERBERT. Department of Zoology and Entomology, Ohio State University, Columbus, Ohio.
- OSBURN, RAYMOND C. Department of Zoology and Entomology, Ohio State University, Columbus, Ohio.
- OTT, GEORGE L. Western Avenue, Cedarsburg, Wis.

- OTTO, GILBERT F. Department of Parasitology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Md.
- OWEN, WILLIAM B. Department of Zoology, University of Wyoming, Laramie, Wyo.
- PACKCHANIYAN, ARDZROONY. Department of Preventive Medicine and Public Health, Medical School, University of Texas, Galveston, Tex.
- PALMER, EDDY D. Box 165, 260 Crittenden Boulevard, Rochester, N. Y.
- PARKER, JAMES P. 538 Williams House, West Quadrangle, Ann Arbor, Mich.
- †PARMAN, DANIEL C. Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Box 509, Uvalde, Tex.
- PAUL, ALLARD A. Department of Biology, College of the City of New York, New York, N. Y.
- †PAYNE, GEORGE C. International Health Division, Rockefeller Foundation, Calle Viena 26, Mexico City, Mexico.
- †PEARSE, ARTHUR S. Zoology Department, Duke University, Durham, N. C.
- PENNER, LAWRENCE R. Department of Zoology, University of Connecticut, Storrs, Conn.
- PEQUENO, EDUARDO A. Department of Parasitology, National University of Mexico, Mexico City, Mexico.
- PEREIRA, CLEMENTE. Department of Parasitology, Instituto Biológico de Sao Paulo, Sao Paulo, Brazil.
- PEREZ-VIGUERAS, ILDEFONSO. Avenida 7a #65, Entre lly 2, Ampliacion de Almenares, Marianao, Cuba.
- PESIGAN, TRINIDAD F. Department of Parasitology, Institute of Hygiene, University of Philippines, Manila, P. I.
- PHILIP, CORNELIUS B. Army Medical School, Army Medical Center, Washington, D. C.
- PIFANO, C. FELISE. Instituto Nacional de Higiene, Caracas, Venezuela.
- PIGMAN, ELBRIDGE G. U. S. Bureau of Animal Industry, Route 3, Box 932, Houston, Tex.
- PIPKIN, COLLINS A. Department of Tropical Medicine, Tulane School of Medicine, New Orleans, La.
- PITERNICK, GEORGE. Department of Zoology, University of California, Berkeley, Calif.
- POMRENKE, KARL R. Department of Zoology, University of Illinois, Urbana, Ill.
- PORTER, ANNIE. Zoological Society of London, Regent's Park, London, N.W.8, England.
- PORTER, DALE A. Regional Laboratory, U. S. Bureau of Animal Industry, Auburn, Ala.
- POTE, THOMAS B. Department of Pathology, Washington University Medical School, St. Louis, Mo.
- PRICE, DAVID. 600 West 165th Street, New York, N. Y.
- †PRICE, EMMETT W. Zoological Division, U. S. Bureau of Animal Industry, Beltsville Research Center, Beltsville, Md.
- RANKIN, JOHN S., JR. Zoology Department, University of Washington, Seattle, Wash.
- RAPPAPORT, IRVING. Department of Public Health, Cornell University Medical College, New York, N. Y.
- RATCLIFFE, HERBERT L. Department of Comparative Pathology, Medical School, University of Pennsylvania, Philadelphia, Penn.
- REARDON, LUCY. 805 Prince Street, Alexandria, Va.
- REDMOND, WILLIAM B. Department of Biology, Emory University, University, Ga.
- REES, CHARLES W. Division of Zoology, National Institute of Health, Bethesda, Md.
- REIBER, ROBERT J. Department of Zoology, University of Georgia, Athens, Ga.
- REID, W. MALCOLM. Department of Biology, Monmouth College, Monmouth, Ill.
- REMLEY, LESLIE W. Oak Ridge Avenue, La Grange, Ill.

- RENDTORFF, ROBERT C. Department of Epidemiology, School of Public Health, Ann Arbor, Mich.
- †REYNOLDS, BRUCE D. Department of Biology, University of Virginia, Charlottesville, Va.
- †RHODES, ROBERT C. Department of Biology, Emory University, University, Ga.
- RHUDE, KENNETH I. Department of Tropical Medicine, Tulane School of Medicine, New Orleans, La.
- RICHARDSON, LAURENCE R. Department of Biology, Victoria University College, Wellington, W 1, New Zealand.
- †RIETZ, JOHN H. West Virginia Experiment Station, Morgantown, W. Va.
- †RILEY, WILLIAM A. Division of Entomology and Economic Zoology, University of Minnesota, Minneapolis, Minn.
- RISER, NATHAN W. 1908 East 27th Street South, Salt Lake City, Utah.
- RITCHIE, LAWRENCE S. Department of Biology, Woman's College of University of North Carolina, Greensboro, N. C.
- ROBERTS, RAIFORD A. 1104 South Runnels Street, Big Spring, Tex.
- RODANICHE, ENID C. Department of Medicine, University of Chicago, Chicago, Ill.
- ROTHMAN, MAURICE M. Department of Gastroenterology, University of Pennsylvania, Philadelphia, Penn.
- ROUDABUSH, ROBERT L. Ward's Natural Science Establishment, Rochester, N. Y.
- ROZEBOOM, LLOYD E. Department of Parasitology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Md.
- ROZYCKI, ANTHONY T. Department of Zoology, University of Minnesota, Minneapolis, Minn.
- RUHE, DAVID S. 1114 Webster Street, New Orleans, La.
- RUTHERFORD, ROBERT L. Departments of Histology, Pathology, and Anatomy, University of Southern California Dental College, Los Angeles, Calif.
- SALSBUURY, JOHN G. Charles City, Iowa.
- SAMORODIN, ALBERT J. 183 Pinehurst Avenue, New York, N. Y.
- †SANDGROUND, JACK H. The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Ind.
- SARLES, M. P. Zoological Division, U. S. Bureau of Animal Industry, Beltsville Research Center, Beltsville, Md.
- †SAUNDERS, LESLIE G. Department of Zoology, University of Saskatchewan, Saskatoon, Canada.
- SAWITZ, WILLI. Department of Tropical Medicine, Tulane University, New Orleans, La.
- †SAWYER, WILBUR A. International Health Division, Rockefeller Foundation, 29 Ferndale Drive, Hastings-On-Hudson, N. Y.
- SCHEFF, GEORGE J. 260 Sheffield Avenue, New Haven, Conn.
- †SCHELL, MARGARET W. Department of Zoology, University of Utah, Salt Lake City, Utah.
- SCHNEIDER, MORRIS D. Headquarters 100th Infantry Division, Fort Jackson, S. C.
- †SCHWARTZ, BENJAMIN. Zoological Division, U. S. Bureau of Animal Industry, Washington, D. C.
- †SCOTT, J. ALLEN. State Department of Public Health, Atlanta, Ga.
- †SCOTT, JOSEPH M. Department of Biology, Mount Union College, Alliance, Ohio.
- †SCOTT, JOHN W. University of Wyoming, Laramie, Wyo.
- SEAMSTER, AARON. Northeast Junior College, Monroe, Louisiana.
- SEMRAD, JOSEPH E. Department of Biology, Loyola University, Chicago, Ill.
- SHELANSKI, HERMAN A. Department of Zoology, University of Pennsylvania, Philadelphia, Penn.
- SHELDON, ALBERT J. Johns Hopkins Hospital, Baltimore, Md.
- SHEPARD, WAYNE. R.F.D. No. 1, Atoka, Okla.
- †SHIELDS, RANDOLPH J. School of Medicine, Cheeloo University, Tsinan, Shantung, China.

- SIMMS, BENNETT T. Regional Laboratory, U. S. Bureau of Animal Industry, Auburn, Ala.
- SINGER, ARNOLD J. 45 Shanley Ave., Newark, N. J.
- †SMITH, SEPTIMA. Department of Zoology, University of Alabama, University, Ala.
- †SOMMERMEYER, VIOLA. 301 Medico-Dental Building, San Diego, Calif.
- SPINDLER, LLOYD A. Zoological Division, U. S. Bureau of Animal Industry, Beltsville Research Center, Beltsville, Md.
- SPRAGUE, VICTOR. 1051 Madison Avenue, Huntington, W. Va.
- SPURLOCK, GLENWOOD M. 911 West Moreland Street, Phoenix, Ariz.
- STABLER, ROBERT M. Department of Zoology, University of Pennsylvania, Philadelphia, Penn.
- †STAFFORD, ETHELBERG W. Department of Zoology and Entomology, Mississippi State College, State College, Miss.
- STAGE, HARRY H. Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Portland, Ore.
- STAUBER, LESLIE A. Oyster Investigation Laboratory, New Jersey Agricultural Experiment Station, Port Norris, N. J.
- †STEINER, GOTTHOLD. Division of Nematology, U. S. Bureau of Plant Industry, Washington, D. C.
- †STEWART, M. A. University of California, 112 Agricultural Hall, Berkeley, Calif.
- †STOLL, NORMAN R. Department of Animal and Plant Pathology, Rockefeller Institute for Medical Research, Princeton, N. J.
- †STUNKARD, HORACE W. Department of Biology, New York University, New York, N. Y.
- SUMMERS, WILLIAM A. Bureau of Laboratories, Jacksonville, Fla.
- SVENSSON, RUTH M. Ullraher, Uppsala Friedenreich, Sweden.
- SWANSON, LEONARD E. Florida Agricultural Experiment Station, Gainesville, Fla.
- SWARTZWELDER, JOHN C. Louisiana State University, School of Medicine, New Orleans, La.
- SWEIBEL, VIVIAN. 345 Natural History Building, University of Illinois, Urbana, Ill.
- SWEZY, WILLIAM W. Department of Biology, Defiance College, Defiance, Ohio.
- TALBOT, S. BENTON. Department of Biology, Davis and Elkins College, Elkins, W. Va.
- †TALIAFERRO, WILLIAM H. Department of Bacteriology and Parasitology, University of Chicago, Chicago, Ill.
- TENBROECK, CARL. Department of Animal and Plant Pathology, Rockefeller Institute for Medical Research, Princeton, N. J.
- TERZIAN, LEVON A. 6A Wynnewood Park Apartments, Wynnewood, Penn.
- TETLEY, JOHN H. Massey Agricultural College, Palmerston North, New Zealand.
- †THOMAS, LYELL J. Department of Zoology, University of Illinois, Urbana, Ill.
- THRELKELD, WILLIAM L. Virginia Agricultural Experiment Station, Blacksburg, Va.
- TIDD, WILBUR M. Zoology Department, Ohio State University, Columbus, Ohio.
- TOBIE, JOHN E. Department of Tropical Medicine, Tulane University, New Orleans, La.
- TODD, ARLIE C. Department of Zoology, Louisiana State University, University Station, Baton Rouge, La.
- TOWNSEND, ELSIE W. Department of Biology, Wayne University, Detroit, Mich.
- TRAGER, WILLIAM. Department of Animal and Plant Pathology, Rockefeller Institute for Medical Research, Princeton, N. J.
- TRAVIS, BERNARD V. U. S. Department of Agriculture, Box 655, New Smyrna, Fla.
- TSUCHIYA, HIROMU. Department of Bacteriology and Hygiene, School of Medicine, Washington University, St. Louis, Mo.
- TUCKER, HAL. Medical School, University of Southern California, Los Angeles, Calif.

- †TYZZER, ERNEST E. Department of Comparative Pathology, Harvard Medical School, Boston, Mass.
- †VANCLEAVE, HARLEY J. Department of Zoology, University of Illinois, Urbana, Ill.
- VAN DEN BERGHE, LOUIS. Institut de Medecine Tropicale Prince Leopold, 155 Rue Nationale, Auvers, Belgium.
- VAN VOLKENBERG, HORATIO L. 807 East 29th Street, Bryan, Tex.
- VARGAS, LUIS. National Faculty of Medicine, Calle de la Escuela, Medico-Militar No. 20, Mexico City, Mexico.
- VAUGHN, CHARLES M. Department of Zoology, University of Wisconsin, Madison, Wis.
- VENARD, CARL E. Department of Zoology and Entomology, Ohio State University, Columbus, Ohio.
- VERGEER, TEUNIS. Department of Biology, Hope College, Holland, Mich.
- VOGEL, HANS. Tropeninstitut, Bernard Noehstrasse 74, Hamburg 4, Germany.
- VOGELSANG, ENRIQUE G. Veterinary High School of Caracas, Este 14, No. 66, Caracas, Venezuela.
- VOLK, JOSEPH J. 5931 West Michigan Street, Milwaukee, Wis.
- VON BRAND, THEODOR. Department of Biology, Catholic University of America, Washington, D. C.
- WALKER, J. HENRY. Department of Zoology, University of Alabama, University, Ala.
- WALLACE, FRANKLIN G. Department of Zoology, University of Minnesota, Minneapolis, Minn.
- WALLACE, HAROLD E. McKendree College, Lebanon, Ill.
- †WALTON, ARTHUR C. Department of Biology, Knox College, Galesburg, Ill.
- WANTLAND, WAYNE W. Division of Biological Science, Stephens College, Columbia, Mo.
- WARD, HELEN L. Department of Biological Sciences, Lindenwood College, St. Charles, Mo.
- *†WARD, HENRY B. Department of Zoology, University of Illinois, Urbana, Ill.
- WARD, JAMES W. Department of Zoology and Entomology, Mississippi State College, State College, Miss.
- WARE, ROBERT E. Clemson Agricultural and Mechanical College, Clemson, S. C.
- †WARREN, ANDREW J. International Health Division, The Rockefeller Foundation, New York, N. Y.
- WARREN, HERBERT S. Department of Anatomy, Hahnemann Medical College, Philadelphia, Penn.
- WEATHERS, CURTIS L. Department of Biology, Long Island University, Brooklyn, N. Y.
- WATT, JOHN Y. C. Department of Public Health and Preventive Medicine, Cornell University Medical School, New York, N. Y.
- WEATHERSBEE, ALBERT A. Roosevelt Road, Ensenada Honda, Puerto Rico.
- WEBSTER, J. DAN. Biology Department, Rice Institute, Houston, Tex.
- †WEHR, EVERETT E. Zoological Division, U. S. Bureau of Animal Industry, Beltsville Research Center, Beltsville, Md.
- WEINMAN, DAVID. Department of Clinical Pathology, Harvard University Medical School, Boston, Mass.
- WEINSTEIN, PAUL P. Parasitology Laboratory, State Board of Health, Jacksonville, Fla.
- WELLER, THOMAS H. 1130 Fair Oaks, Ann Arbor, Mich.
- †WENRICH, DAVID H. Zoology Department, University of Pennsylvania, Philadelphia, Penn.
- WERBY, HELEN J. Department of Biology, Seattle College, Seattle, Wash.
- WEST, D. EVELYN. State Department of Health, Hartford, Connecticut.
- WEST, EVALINE. (Mrs. William Plucknett) 525 West First Street, Long Beach, Calif.

- WETZEL, RUDOLF. Institute for Veterinary-Medical Parasitology and Zoology, Luisenstrasse 56, Berlin, Germany.
- WHARTON, GEORGE W., JR. Zoology Department, Duke University, Durham, N. C.
- WHITE, FRANCIS M. Biology Department, Purdue University, West Lafayette, Ind.
- WHITENER, PAUL D. Biology Department, Cramerton High School, Cramerton, N. C.
- WHITLOCK, JOHN H. Department of Veterinary Pathology, Kansas State College of Agriculture and Applied Science, Manhattan, Kans.
- †WHITMORE, EUGENE R. Department of Parasitology and Pathology, Georgetown University, Washington, D. C.
- WICHTERMAN, RALPH. Department of Biology, Temple University, Philadelphia, Penn.
- WILHELM, RAYMOND W. Department of Zoology, University of Missouri, Columbia, Mo.
- WILLEY, CHARLES H. Department of Biology, New York University, University Heights, New York, N. Y.
- WILMOTH, JAMES H. Department of Biology, Brooklyn College, Brooklyn, N. Y.
- †WILSON, FRANCIS H. Department of Zoology, Tulane University, New Orleans, La.
- WINFIELD, GERALD F. Magnolia, Miss.
- WOKE, PAUL A. 6126 54th Avenue, Riverdale, Md.
- WOLFSON, FRUMA. Department of Parasitology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Md.
- WOOD, SHERWIN F. Department of Biology, Los Angeles City College, 855 North Vermont Avenue, Los Angeles, Calif.
- WOODHEAD, ARTHUR E. Department of Zoology, University of Michigan, Ann Arbor, Mich.
- WOOLDRIDGE, DOROTHY S. Mississippi State Department of Health, Jackson, Miss.
- WORTH, C. BROOKE. Department of Zoology, Swarthmore College, Swarthmore, Penn.
- WORTHAM, RUBY A. Department of Histology, University of Oklahoma Medical School, Oklahoma City, Okla.
- WOTTON, ROBERT M. Middlesex University Medical School, Waltham, Mass.
- WRIGHT, WILLARD H. Division of Zoology, National Institute of Health, U. S. Public Health Service, Bethesda, Md.
- YOKOGAWA, S. Department of Experimental Pathology and Parasitology, Taihoku Imperial University, Taihoku, Formosa, Japan.
- YOUNG, BENJAMIN P. Department of Zoology, Cornell University, Ithaca, N. Y.
- YOUNG, MARTIN D. U. S. Public Health Service, Box 1344, Columbia, S. C.
- YUTUC, LOPE M. College of Veterinary Science, Manila, P. I.
- ZAIMAN, HERMAN. Crompton, New York.
- ZARAFONETIS, CHRIS J. D. Army Medical School, Army Medical Center, Washington, D. C.
- ZELIFF, C. COURSON. Department of Zoology, Pennsylvania State College, State College, Penn.
- †ZETEK, JAMES. Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Balboa, Canal Zone.
- ZUCKERMAN, AVIVAH. Department of Bacteriology and Parasitology, University of Chicago, Chicago, Ill.

The Journal of Parasitology

Volume 28

DECEMBER, 1942

Number 6

THE EFFICIENCY OF THE BAERMANN APPARATUS IN THE RECOVERY OF LARVAE OF *HAEMONCHUS CONTORTUS*

A. G. DINABURG

Zoological Division, U. S. Bureau of Animal Industry

The apparatus commonly used for the isolation of nematode larvae from soil was first described by Baermann in 1917. It consisted of a glass funnel closed at the bottom by a clamp placed on a piece of rubber tubing attached to the shank, and a sieve of 1 mm mesh wire covered with a piece of coarse cloth. In order to isolate hookworm larvae from fecal cultures or contaminated soil, the funnel was filled nearly to the brim with filtered and sterilized water and the cloth covered screen containing soil or feces with hookworm larvae was placed in the water in the funnel. Samples of varying sizes were withdrawn from the bottom of the funnel at different intervals and examined for larvae. The temperature of the water was not stated. Cort, Ackert, Augustine and Payne (1922) tested the effect of various changes in the operation of the apparatus upon the recovery of hookworm larvae from soil. They found that the water must be at least 10° F warmer than the soil, that a higher percentage of larvae can be isolated from coarse than from finely divided soils, that a slightly higher percentage of larvae can be isolated from moist than from saturated soil, and that while most of the larvae escape from the soil into the water in the first 6 hours, an appreciable number come out later. They also found that under favorable conditions the percentage of larvae recovered was high but variable, the average being about 52 per cent. Stoll (1923) added known numbers of hookworm larvae to 100 gm samples of different types of soil, and used the Baermann apparatus under the conditions standardized by Cort et al (1922) to recover the larvae. He found that about 90 to 95 per cent of the larvae were recovered from "humus," consisting mainly of vegetation in various stages of decay, about 80 per cent from sand and loam, and about 33 to 50 per cent from clay. Cort, Stoll, and Grant (1926) used water routinely at 45° C, after having found that of 1900-3750 mixed *Ancylostoma* and *Necator* larvae placed on 200 gram lots of moist clay-loam soil, and allowed to remain there

Received for publication, December 30, 1941.

about 24 hours before test isolation, an average of 58.1 ± 1.6 per cent were recovered from Baermann units set up with water at 30–50° C and allowed to stand over night before being drawn off. This was in mid-summer at Soochow, China, with air temperatures 27–32° C.

Mönnig (1930) "made critical tests with this method on *Trichostrongylus*, *Haemonchus contortus*, and *Oesophagostomum columbianum* larvae in fecal cultures and in different types of soil and found that it gave excellent results, comparable to those obtained by Cort et al (1922), with hookworm larvae. The best results were obtained with the coarsest soils and a small apparatus, using funnels of 6-inch diameter."

Parnell (1936) added known numbers of horse strongyle larvae previously isolated by the Baermann apparatus to 40 gm of sterilized horse feces suspended in a cheesecloth bag in a funnel of water. A 50-cc sample was then examined for the number of larvae recovered. When the initial number of larvae was 75 to 100, percentages of 12.5, 25, and 45 were recovered from 3 cultures, respectively. When the number of larvae was 700 to 1,000, percentages of 35, 35, and 50, respectively, were recovered, and when the original number was about 10,000 the recoveries were 45, 65, and 90 per cent, respectively.

Kauzal (1940) tested the effect of various modifications of the Baermann technic on its efficiency for the recovery of infective larvae of *Haemonchus contortus* and *Trichostrongylus* spp. Either the former species or both were used, but the results are not given separately, nor are the methods of obtaining the larvae given. He found that more larvae were recovered when water at room temperature (15° to 22° C) was used than when water at 37° C was put into the funnel. The soil plots containing the larvae were supported on wire gauze in funnels of various sizes. With a 6-inch funnel and 50 gm soil, the average recovery in 11 experiments was 33.6 per cent, while 17 per cent was recovered when 100 gm soil in the same size funnel were used. With a 9-inch funnel and 100 gm soil, 43 per cent were recovered. These tests were carried out under identical conditions, with water at room temperature, and an isolation period of 48 hours. However, the numbers of larvae involved are not given. In other experiments, Kauzal found that when the soil was supported by wire gauze the mean recovery was 36.7 per cent; when no gauze was used it was 58.3 per cent. He concludes that the percentage of larvae recovered by this method is low and varies greatly in individual experiments.

PURPOSE OF EXPERIMENTS

The Baermann apparatus was used regularly to recover larvae of *Haemonchus contortus* from stirred samples of soil, sheep feces, and mixtures of both in experiments on the development and survival of the eggs and larvae of this parasite under outdoor conditions. In view of

the extensive use of the apparatus in the above experiments, and the scarcity of available information on the efficiency of the apparatus in the recovery of larvae of *H. contortus*, the following experiments were performed to calibrate the apparatus under conditions¹ similar to those used in the survival experiments.

MATERIALS AND METHODS

The Baermann apparatus consisted of glass funnels provided with rubber tubes, and clamps at the lower end; of straight-sided screens; and of heavy muslin cloths. The funnels were $7\frac{5}{8}$ inches in diameter across the top; the screens, $7\frac{1}{16}$ inches outside diameter and $2\frac{3}{4}$ inches high, and were made of 16-mesh wire screen. The cloths were washed muslin about 35 cm square and weighed about 25 gm. After each experiment the screens and funnels were first washed, and then dipped into boiling water; the cloths were washed, boiled and dried before using in order to kill any remaining larvae.

The procedure used in each experiment was as follows: Six funnels were filled with water at 40° C, to a level below that of the screens. The sterilized cloths were put in each of 6 screens. One hundred grams of steam-sterilized sandy loam was put into each of 2 screens; and in each of 2 other screens, 100 gm ground sheep feces, free of nematode eggs, except for occasional *Strongyloides papillosus* eggs, while in the third pair, neither soil nor feces was placed. The suspension of infective larvae, collected the day of the experiment from cultures of *H. contortus* eggs, was stirred well before each sampling, and 8 or 10 samples of equal volume drawn up with a pipette. Two of the samples were put into separate bottles as controls and 6 samples were pipetted slowly into each of the screens. If sufficient suspension remained, two more control samples were taken. The water level was then brought to just below the surface of the soil or feces, or above that of the cloth, and the ends of the cloth tucked in. Under these conditions, the funnels contained about 1600 cc of water. After the larvae had been in the apparatus for 48 hours a sample of 50 to 60 cc was drawn from the bottom of each funnel by pinching the rubber tube above the clamp with metal tongs, while loosening the clamp, then relaxing the pressure to flush the whole tube.

The number of larvae in each sample and those in the control bottles were determined by examination either of the whole sample or of a diluted part of it under a dissecting microscope. In most experiments 4 control samples were available; at least 4, and in most cases 5, dilution counts were made on each control, and on each Baermann sample containing large numbers of larvae.

¹ The specifications of the components of the apparatus, initial temperature of the water, length of isolation period, and weight of the soil sample used in these experiments were suggested by Dr. D. A. Shorb of this laboratory.

When large numbers of larvae were available, several experiments with different numbers of larvae were set up at the same time.

RESULTS OF EXPERIMENTS

The results of 27 experiments are graphed in Fig. 1. Because of the wide range of numbers involved a logarithmic scale was used for the numbers of larvae put into the apparatus. The percentage recovered

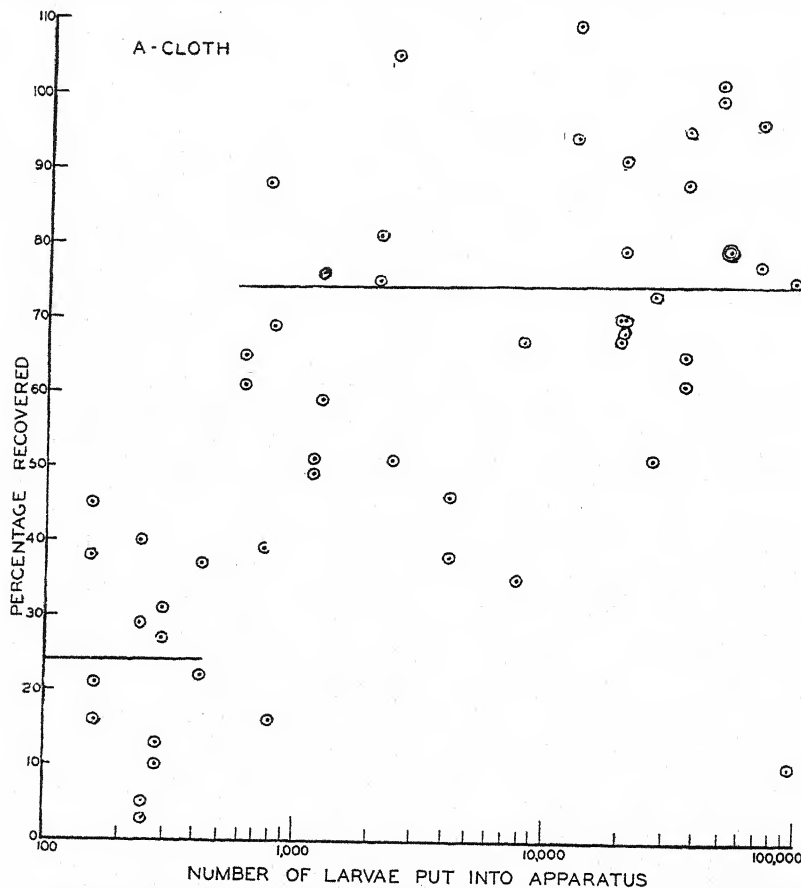


Fig. 1 A. Percentage of infective larvae of *H. contortus* recovered after passage through cloth in the Baermann apparatus.

was obtained by dividing the number recovered from each unit by the average of the numbers in the control samples. In one experiment with 12,600 larvae, there were enough larvae for only one soil unit, from which 60 per cent were recovered. Because of the extensive comparisons involving duplicates, the data for this single unit was omitted.

The data in Fig. 1 indicate that with an input of less than 600 larvae, the percentages recovered after passage through cloth and through soil

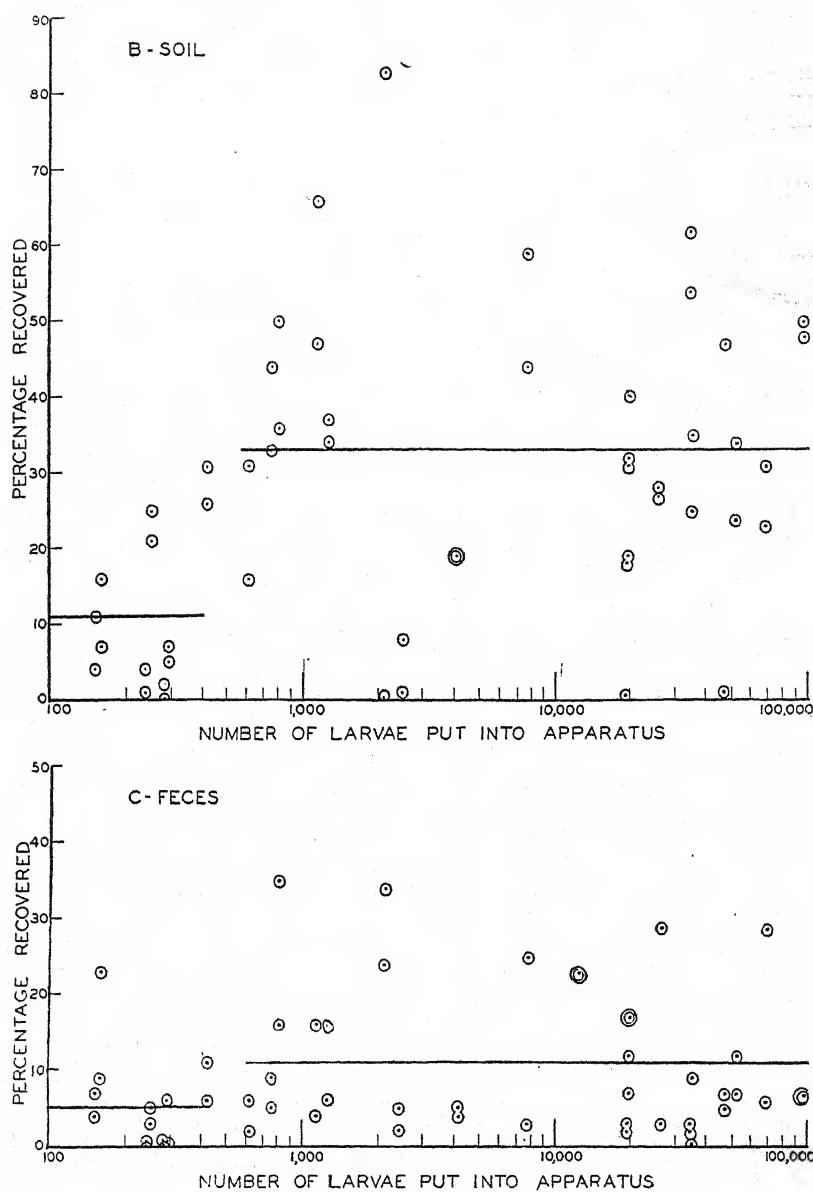


Fig. 1 B, C. Percentage of infective larvae of *H. contortus* recovered after passage through cloth plus 100 gm of soil (B) and cloth plus 100 gm of ground sheep feces (C) in the Baermann apparatus.

are not in line with those recovered from higher inputs. For this reason, the results of 7 experiments with inputs below 600 larvae were treated separately from the results of 20 involving more than 600 larvae.

In the 7 experiments involving inputs of 150 to 420 larvae, the percentage recovered through cloth, soil, and feces averaged 24, 11, and 5, and ranged from 3 to 45, 0 to 31, and 0 to 23 per cent, respectively. One soil unit and 2 feces units did not yield any larvae.

In the experiments with inputs ranging from 600 to 95,400 larvae, the averages of the percentages recovered through cloth, soil and feces were 69, 34 and 11 per cent, respectively. Analyses of variance (Snedecor, 1940) on the percentages recovered in each of the three groups showed that the average percentages differed significantly from each other, but that within each of the groups the variance between experiments were not significantly greater than that within experiments. This second test gave a mean square for variation between duplicates that was about $2/3$ that from the variation between experiments in the same group. This would indicate almost as large a variation between duplicates as between experiments, as is also indicated by the data in Fig. 1. However, closer examination of the data showed that 5 pairs of units in the cloth series differed by more than 22 per cent in their percentage recovery, 2 pairs in the soil series, and 1 pair in the feces series. None of these pairs were found in the same experiments, or at one end of the input range. However, 3 pairs in the cloth series occurred with inputs below 2,500 larvae, and 4 with inputs below 8,000 larvae.

If the data from these 8 pairs are set aside, the average percentage recovery is not changed materially, but new information about the operation of the apparatus can be obtained from the data from the remaining 51 pairs. The new averages of the percentage recoveries for cloth, soil, and feces now become 74, 33, and 11 per cent, respectively. An analysis of variance of the percentages in these reduced groups is shown in Table 1.

TABLE 1.—*Analysis of variance of percentages of larvae recovered by the Baermann apparatus, after passage through various materials*

Material	Source of variation	Degrees of freedom	Variance
Cloth	Between experiments	14	572**
	Between duplicates	15	69
Cloth plus soil	Between experiments	16	470**
	Between duplicates	17	60
Cloth plus feces	Between experiments	18	129*
	Between duplicates	19	50

* Significant difference, by F test.

** Highly significant difference, by F test.

This analysis indicates that the percentages recovered in the successive experiments differ significantly more than do duplicates within the same experiment. From this analysis the variance due to these two factors can be isolated. The variances due to differences between duplicates for the cloth, soil, and feces series are 69, 60, and 50, while the variances due to

differences between experiments are 251, 205, and 40, respectively. Interpreted in terms of the operation of the apparatus, these figures indicate that the variations in the experimental technics before and after the larvae have been in the apparatus, are about the same in the three groups of experiments. The differences between the experiments, after the separation of this experimental error, are much larger than this source of variation in the cloth and soil series, and are due to the inconsistent operation of the apparatus from one experiment to the next. No regular variation of percentage recovery with size of input could be found.

To complete the account of the data, the average recoveries of the 8 pairs of units set aside were 55, 33, and 16 per cent for the cloth, soil, and feces series, respectively. In the cloth series, the variance between experiments was less than that within experiments. No explanation for the results in this group of 8 pairs can be given.

DISCUSSION

The data indicate that the variability in the performance of the apparatus is much more responsible than the variability in the other experimental technics for the differences between experiments with either cloth or soil when more than 600 larvae were put into the apparatus. In experiments with layers of feces these two factors are about equal, perhaps because of the low upper limit on the percentages recovered set by the bulk of material. The reasons for the variability in the performance of the apparatus were not investigated, since the present experiments were not designed for that purpose. However, it has been possible to isolate the effect of the apparatus from the effect of the other technics involved in using the apparatus, and to measure the average percentage recovery of the larvae of *H. contortus* through various materials.

These results bear directly on the interpretation of recoveries of infective larvae of *H. contortus* with the Baermann apparatus, under conditions similar to those of the present experiments. Considering the low average recoveries through 100 gms of soil or feces with inputs of 150 to 420 larvae, and with occasional failure to recover any larvae, it would appear advisable to weigh carefully low or negative recoveries with the apparatus when the initial number in the sample is expected to be low. Even when the initial number is expected to be much larger, the effect of the type of substrate and of the variability of the apparatus upon the net efficiency of the apparatus should be considered in dealing with the numbers of larvae recovered.

SUMMARY

1. The percentage of infective larvae of *H. contortus* recovered after passage in the Baermann apparatus through cloth, cloth plus 100 grams

of sandy loam, and cloth plus 100 grams of ground sheep feces, was determined in 27 experiments.

2. The efficiency of the Baermann apparatus varied with the type of material used as substrate and the initial number of larvae. In 7 experiments involving 150 to 420 larvae, an average of 24, 11, and 5 per cent were recovered after passage through layers of cloth, soil, and feces, respectively, whereas in 15 to 19 experiments with 600 to 95,400 larvae, these averages were 74, 33, and 11 per cent, respectively.

3. In experiments with more than 600 larvae, the variations in percentage recovery between experiments with cloth or soil layers were due mainly to inconsistent operation of the apparatus, rather than to variations in the technic of handling and counting of the larvae before and after passage through the apparatus. The variation in technic is about the same in all three groups.

BIBLIOGRAPHY

- BAERMANN, G. 1917 Eine einfache Methode zur Auffindung von Ankylostomum (Nematoden) Larven in Erdproben. *Geneesk. Tijdschr. Nederl.-Indie* 57: 131-137.
- CORT, W. W., ACKERT, J. E., AUGUSTINE, D. L. AND PAYNE, F. K. 1922 Investigations on the control of hookworm disease. II. The description of an apparatus for isolating infective hookworm larvae from soil. *Am. J. Hyg.* 2: 1-16.
- CORT, W. W., STOLL, N. R. AND GRANT, J. B. 1926 Researches on Hookworm in China. I. Problems and methods of attack. *Am. J. Hyg. Monogr. Ser.* 7: 1-32.
- KAUZAL, G. P. 1940 Experiments on the recovery of sheep nematode larvae from pastures. *J. Council Scient. and Indust. Research Australia* 13: 95-106.
- MÖNNIG, H. O. 1930 Studies on the bionomics of the free-living stages of *Trichostrongylus* spp. and other parasitic nematodes. *Rep. Director Vet. Serv., Dept. Agric., Union South Africa* 16: 175-198.
- PARNELL, I. W. 1936 Studies on the bionomics and control of the bursate nematodes of horses and sheep. II. Technique. *Canad. J. Research* 14(D): 71-81.
- SNEDECOR, G. W. 1940 *Statistical Methods*. Collegiate Press; Ames, Iowa. 3 ed.
- STOLL, NORMAN R. 1923 Investigations on the control of hookworm disease. XXIV. Hookworm cultures with humus, sand, loam and clay. *Am. J. Hyg.* 3 (July Suppl.): 1-36.

THE EFFECT OF SULFONAMIDE COMPOUNDS ON THE GROWTH OF *ENDAMOEBA HISTOLYTICA* IN CULTURE*

ENID C. RODANICHE AND JOSEPH B. KIRSNER

Department of Medicine, University of Chicago

The sulfonamide compounds have received in general less attention in the therapy of parasitic than of bacterial diseases. A review of the literature reveals only one reference to the use of any of these compounds in amebic infection (Saini, 1940). Since *Endamoeba histolytica* is readily maintained in culture, it was decided to use the culture method to study the action of the sulfonamide compounds on this organism. Sulfanilamide, sulfapyridine, sulfathiazole and sulfaguanidine were given extensive trial. Several experiments were conducted also with sulfadiazine as noted in the text.

Strains of Endamoeba histolytica. The majority of the experiments were made with a strain of *E. histolytica* which had been isolated from the stool of a patient with amebic dysentery 2½ years previously and had been maintained in culture since that time. A few experiments were performed with strains freshly isolated from recent cases and carriers.

Media. For cultivation of *E. histolytica* a slight variation of the Cleveland and Collier (1930) medium was utilized, the variation consisting in the use of one part of sheep serum and 7 parts of Locke's solution without dextrose in place of the usual serum and physiological salt solution to make up the fluid portion of the medium. Very heavy growth has been obtained consistently in this medium with stock strains. Growth with old strains usually reaches a maximum in 3 days with subsequent decline and death in 5 to 9 days. With freshly isolated strains this process is usually accelerated.

As is well known *E. histolytica* cannot be maintained in culture in the absence of bacteria. In fact, the type and quantity of the accompanying bacteria or fungi profoundly affects the success or failure of cultivation. Faust (1941) has suggested that some type of symbiotic relationship is involved. Our stock culture has become stabilized and uniformly shows growth of coliform bacilli, alpha and gamma streptococci and staphylococci. More recently isolated strains present a wider variation in the bacterial flora. Such a relationship of course complicates the evaluation of the effect of the drugs, but may present a truer picture of the probable action of these compounds in the body.

Received for publication, January 21, 1942.

* This work was done under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

Sulfonamide Compounds. Saturated solutions of the sulfonamide compounds were prepared by adding an excess of these substances to the Locke's solution-serum mixture. The mixture was then placed in the incubator at 37.5° C for 2 hours and the excess drugs removed by filtration through a Berkefeld N filter; this process also served to sterilize the filtrate. Exactly 5 cc of the fluid was then used to overlay the liver infusion agar slants.

For some experiments a 3 mm loopful of the respective crystalline compounds was added to the saturated drug slants. No effort was made to sterilize the drugs before addition. However, it was not found that the contamination so introduced produced any significant alteration in the cultures.

The initial experiments were designed to give a general idea of the action, if any, of the sulfonamide compounds on the amebae. For this purpose, four tubes containing respectively sulfanilamide, sulfathiazole, sulfaguanidine, and sulfapyridine in solution were inoculated and incubated at 37.5° C. A control tube without drugs was similarly treated. The cultures were examined periodically to determine the presence or absence and the relative abundance of the amebae by transferring a small amount of the sediment to a glass slide and examining under the microscope. The results are shown in Table 1. The amebae were found to

TABLE 1.—Effect of the addition of sulfonamide compounds in saturated solution on the life of *Endamoeba histolytica* in culture

Compound added in saturated solution	Life of culture in days							
	1	3	5	7	10	12	15	20
Sulfanilamide	P	P	0	0	0			
Sulfaguanidine	P	P	P	P	0	0		
Sulfathiazole	P	P	P	P	P	P	0	0
Sulfapyridine	P	P	P	P	0	0		
Control	P	P	P	0	0			

P indicates that living forms were present.
0 indicates that no living forms were observed.

survive in the saturated sulfanilamide preparation for 3–5 days, in sulfaguanidine for 7–10 days, in sulfathiazole for 12–15 days, in sulfapyridine for 7–10 days, and in the control for 5–7 days. In general growth was less abundant in the cultures containing the drugs than in the control and was particularly scanty in the tube containing sulfanilamide. This preliminary work established the fact that *E. histolytica* was able to survive in cultures containing saturated solutions of three sulfonamide compounds as long or longer than in controls. The growth of this organism, however, was diminished.

The question then arose as to whether the survival of *E. histolytica* was due to a gradual diminution in the activity of the sulfonamide drugs with the passage of time. To study this factor culture tubes prepared as

previously described but containing a loopful of the crystalline compounds were employed. Since the crystals could be readily identified by examination of the sediment, one could be certain that the drugs remained in excess throughout the observation period.

The results of this experiment appear in Table 2. It will be noted

TABLE 2.—*Effect of the addition of sulfonamide compounds in the crystalline state on the persistence of Endamoeba histolytica in culture*

Compound added in crystalline form	Life of culture in days									
	1	3	5	7	10	12	15	20	24	26
Sulfanilamide	0	0	0							
Sulfaguanidine	P	P	P	P	P	P	0	0		
Sulfathiazole	P	P	P	P	P	P	P	P	P	0
Sulfapyridine	P	P	P	P	P	0	0			
Control	P	P	P	0	0					

P indicates that living forms were present.
0 indicates that no living forms were observed.

that, with the exception of sulfanilamide, growth persisted in the presence of the crystalline drugs for periods longer than were noted with the saturated solutions and for a much longer time than was observed in the control. In the culture containing crystals of sulfanilamide, however, growth had ceased within 24 hours. The amebae showed neither positive nor negative chemotaxis toward the sulfonamide crystals, progressing towards, among and away from them freely. It was noted again, however, that growth in the cultures containing the drugs was less abundant than in the control.

Having established by this preliminary work that amebae will survive, though in diminished numbers, in saturated and supersaturated solutions of sulfapyridine, sulfathiazole and sulfaguanidine and in saturated solutions of sulfanilamide, our next step was to apply a more accurate quantitative method to determine the actual degree of inhibition. For this purpose, the test and control cultures were inoculated with exactly 0.1 cc of an evenly dispersed suspension of *E. histolytica*. At various periods the fluid contents of the tubes were well stirred, and 0.05 cc quantities were removed to slides, covered with cover-glasses, and the total number of amebae in 16 fields taken along the diagonals at regular intervals was counted under the low power of the microscope. The average number of organisms per field was derived from this procedure. The entire preparation was first examined rapidly to make certain there was even dispersion and no clumping. Careful check indicated that this method gave a sufficiently accurate picture of the relative abundance of the organisms. Examinations were made at 1, 2, 3, 5, 6, 7, 10, 17, 24 and 27-day intervals. Growth was so scanty after an interval of 17 or more days that counts were not made. The whole preparation was examined, however, and if any viable organisms were

detected, the culture was considered positive. These results appear in Table 3. Reference to this table establishes two major findings:

TABLE 3.—*Effect of saturated solutions of sulfonamide compounds on the growth of Endamoeba histolytica and accompanying bacteria in culture*

Compound added in saturated solution		Average number of amebae and bacteria in culture after varying periods of incubation*										
		Days										
		1	2	3	5	6	7	10	17	24	27	
Sulfanilamide	Amebae	0.25	0.1	0	0							
	Bacteria	680M	380M	250M			90M					
Sulfaguanidine	Amebae	4.1	30.7	37.0	16.0	10.0	9.0	0	0			
	Bacteria	920M	370M	400M			57M					
Sulfapyridine	Amebae	6.0	18.9	49.2	13.5	11.9	9.9	0	0			
	Bacteria	1B	640M	590M			170M					
Sulfathiazole	Amebae	7.9	11.5	16.9	5.5	3.2	1.6	4.0	P	P	0	
	Bacteria	950M	520M	410M			90M					
Control	Amebae	17.9	63.4	64.4	0	0						
	Bacteria	1B	930M	760M			150M					

* Number of amebae equals the average count per 16 fields.
 Number of bacteria equals the number per cc of culture fluid.
 B indicates billion.
 M indicates million.
 P indicates living amebae were present.
 0 indicates no living amebae were found.

1. All the sulfonamide compounds tested produced inhibition of the growth of *E. histolytica* in culture. This inhibition was most marked in the case of sulfanilamide, followed in order by sulfathiazole, sulfaguanidine and sulfapyridine.

2. Viable amebae persisted in culture for much longer periods of time when the drugs other than sulfanilamide were present than in the control preparations. Living amebae disappeared from the cultures which were not drug-treated between the 3rd and 6th day, whereas they persisted in the cultures containing sulfaguanidine, sulfapyridine and sulfathiazole from 9 to 24 days. The fact that viability was maintained for longer periods in this series than in the preliminary experiments is attributable to the fact that these cultures were submitted to thorough agitation at each examination with consequent dispersion of local accumulations of toxic waste products, whereas previously the sediment alone was examined after very little agitation of the supernatant fluid.

It is of interest to note that maximum growth occurred on the third day for all the cultures except that containing sulfanilamide, and was then followed by rapid decline in the control and by gradual decline in the test preparations.

There was no marked morphological change in the drug-treated amebae, except that there was usually prolonged persistence of large forms. Such large forms ordinarily appear in the untreated cultures only during the early growth phase and then are supplanted by smaller forms during the period of maximum growth and decline. This observation

might indicate a prolongation of the growth phase under the action of the sulfonamides. Amebae from both untreated and drug-treated cultures showed the same degree of motility and contained approximately the same amount of ingested starch granules and bacteria. Stained smears of both control and drug-treated amebae showed typical morphology with nuclear divisions appearing in stained individuals in both groups. However, no attempt was made to determine the relative number of fissions occurring among the individuals of the control and test groups. Encystation occurred in the drug-treated cultures as frequently as in the controls, and the cysts appeared typical. It may be stated that encystation is less frequent and more irregular in strains maintained for several passages on artificial media than in freshly isolated strains. No stimulation of encystment, however, was provided by the sulfonamide compounds.

Since, as previously stated, the growth of amebae in culture involves a probable symbiotic relationship with bacteria, the question arises as to what effect the sulfonamides exert on the bacterial flora of the cultures, and to what extent this effect is reflected in the ameba population. To study this problem estimates were made of the relative numbers and types of bacteria present in the various culture tubes. One-tenth cc of the well agitated culture fluid employed for the ameba counts was withdrawn and used to prepare serial dilutions up to 1-10 million in distilled water. These dilutions were then utilized to seed veal infusion agar pour plates which were incubated at 37.5° C for 48 hours. At this time the number of colonies developing per plate was counted and the number of viable organisms per cc of culture fluid thus estimated. Identification of typical colonies was made also by staining, fermentation and other tests. The high dilutions employed were believed to nullify any inhibitory effect of the drugs on the growth of viable organisms in the plates. These results appear in Table 3, and show that the sulfonamides tested exerted only a slight inhibitory effect on the growth of the bacteria. Furthermore, this action did not appear to be selective, for both gram positive cocci and gram negative bacilli persisted. The inhibition of *E. histolytica* in culture apparently, then, cannot be correlated with a simultaneous bacteriostasis.

The above experiment was repeated using culture fluids containing a loopful of the crystalline drug. The method was as previously described except that observations were not made beyond the 17-day period. The results are shown in Table 4. As in the preliminary experiments, amebae disappeared in 24 hours in the presence of crystalline sulfanilamide. Distinct inhibition of growth was produced by the other compounds, although the order of the inhibitory effect was changed, the alteration being most pronounced in the case of sulfaguanidine.

The degree of stasis of amebic growth may coincide to some extent with the solubility of the drugs used: sulfanilamide, which exerted the

TABLE 4.—Effect of supersaturated solutions of the sulfonamide compounds on the growth of *Endamoeba histolytica* in culture

Compound added in crystalline form	Average number of amebae counted in 16 fields after periodic intervals of incubation						
	Days						
	1	2	3	6	7	8	17
Sulfanilamide	0	0	0				
Sulfaguanidine	0.94	2.7	8.2	16.4	20.1	19.6	P
Sulfapyridine	1.9	28.1	12.6	6.0	5.3	3.3	0
Sulfathiazole	0.7	20.1	15.4	6.2	3.8	1.7	P
Control	0.9	40.6	66.6	3.1	2.5	2.1	0
Saturated solution of sulfanilamide ..	1.0		0.2	0		0	
Above solution diluted 1 to 5	4.1		8.0	12.2		1.9	

P indicates living amebae were present.
0 indicates no living amebae were found.

greatest inhibitory effect, is also the most soluble. To further elucidate this question, a saturated solution of sulfanilamide at 37.5° C was diluted 1–5 times with normal serum-Locke's solution and then inoculated with *E. histolytica*. A quantitative estimation of the degree of growth was made over a period of 8 days and the result compared with that observed in a control tube and in a tube containing a saturated solution of sulfanilamide. The results appear in the lower part of Table 4. It will be readily noted that the inhibitory effect of sulfanilamide greatly decreased by dilution of the drug, but that even in this dilution growth was by no means as great as that in the control preparation.

Sulfadiazine. A few tests of the effect of sulfadiazine were made. It was found that *E. histolytica* grew well in culture media containing this compound in saturated and in supersaturated solution. Inhibition was most noticeable in the first two days of culture. On the second day an average count of 2.4 amebae in 16 fields was obtained in the sulfadiazine-saturated preparation, whereas the average count in the control was 15.4. However, growth rapidly increased in the drug-treated culture, and living amebae were still fairly numerous after 11 days of incubation, although life had ceased in the control culture at the end of 7 days. It was very interesting to note that *E. histolytica* readily ingested the smaller crystals of sulfadiazine, a phenomenon not observed with the other sulfonamide compounds, although it may have occurred occasionally. The presence of these ingested crystals did not seem to impair the activity of the amebae during the 5 or 10 minute periods of observation.

To clarify further the action of sulfanilamide on the growth of *E. histolytica*, the following experiment was conducted. Four of the regular culture tubes were inoculated with 0.1 cc of an evenly dispersed suspension of amebae and the tubes were incubated for 24 hours. At the end of this time, the fluid contents were well mixed and the average number of amebae in 16 fields of a slide preparation counted as described previously. The tubes were then centrifuged, the supernatant was discarded and re-

placed as follows: to tube No. 1 was added exactly 5 cc of untreated serum-Locke's solution; to tube No. 2, 5 cc of serum-Locke's solution saturated with sulfanilamide; to tube No. 3, the same plus a loopful of crystalline sulfanilamide; and to tube No. 4, serum-Locke's solution with added sulfanilamide and para-amino-benzoic acid. The tubes were then replaced in the incubator, and microscopic examination with counts of 16 fields performed at the end of 15 minutes, 2½, 5, 24 and 48 hours. Examination without count was also made in 96 hours. These results are recorded in Table 5.

TABLE 5.—*Effect of sulfanilamide on the growth of amebae in culture*

Culture tube No.	Av. no. of amebae after 48 hrs. incubation	Further treatment	Av. no. of amebae after incubation for hours					
			¼	2½	5	24	48	96
1	25.1	Supernatant replaced with untreated serum-Locke's solution	24.7	31.5	66.6	84.2	20.0	P
2	21.4	Supernatant replaced with serum-Locke's solution saturated with sulfanilamide	20.8	15.1	9.5	21.6	13.9	P
3	17.8	Supernatant replaced with serum-Locke's sol. and crystals of sulfanilamide added	15.8	16.3	15.6	6.9	2.4	0
4	16.3	Supernatant replaced with serum-Locke's sol. containing crystals of sulfanilamide and p-amino benzoic acid	11.2	3.3	4.4	4.7	2.7	P

P indicates living amebae were present.
0 indicates no living amebae were found.

It will be noted that the number of amebae remained stationary in all tubes for 15 minutes, except in tube No. 4 in which there was a significant decline in the ameba count. After an interval of 15 minutes the number increased up to the 24-hour period in tube No. 1, declined up to the 5-hour period in tube No. 2 and then rose, remained stationary up to the 5-hour period in tube No. 3 and then declined, and declined steadily in tube No. 4. A "latent period" apparently operates in the inhibition of amebic growth by sulfanilamide as well as in bacteriostasis. It may be noted that life persisted in the preparation containing crystalline sulfanilamide for 2-4 days whereas in previous experiments it had disappeared in 24 hours. This may be attributed to a difference in the preparation of the tubes with a much higher initial inoculum, and also to frequent agitation of the fluid with dispersion of particles. It is of interest that although the addition of p-amino-benzoic acid seemed to precipitate a more rapid decrease in the number of amebae it permitted the persistence of living amebae beyond the period tolerated in the preparation containing crystalline sulfanilamide alone.

Adaptation to Sulfanilamide. An attempt was made to increase the

sulfanilamide tolerance of the amebae by continuous passage in culture media containing this compound. Such an adaptation was readily effected, growth appearing more heavy with each of 4 successive passages. For the fifth passage, the adapted amebae were inoculated into a tube containing crystals of sulfanilamide. The organisms were able to tolerate this treatment for more than 3 days, although growth remained scanty.

Recently Isolated Strains. Two strains freshly isolated from 2 different patients with amebic dysentery were inoculated on the second passage in artificial media into culture tubes containing saturated solutions of sulfanilamide, sulfaguanidine, sulfathiazole and sulfapyridine. Results were in general comparable to those obtained with the old stock strain.

In addition control culture tubes and tubes treated with sulfaguanidine, sulfapyridine and sulfathiazole were seeded with fecal material containing cysts only of *E. histolytica* obtained from a carrier. Excystation and growth occurred in the control preparation and the preparation treated with sulfathiazole in 24 hours. However, in the cultures containing sulfaguanidine and sulfapyridine excystation was delayed for 48 hours. Growth persisted for 1 to 2 days in the drug-treated cultures beyond that in the controls.

Black and his co-workers (1941) have demonstrated that liver extract is more effective than p-amino-benzoic acid in counteracting the action of sulfaguanidine in vivo. Since the culture medium used in the present experiments contained liver infusion agar, the possibility arose that the liver might have diminished the inhibitory effect of the drugs. An experiment was therefore devised, using as a culture medium only serum-Locke's solution, a preparation suitable for the cultivation of amebae as shown originally by Craig (1934). In our experience growth is much scantier and more irregular in this medium than in the Cleveland and Collier medium, in view of which its use has been avoided heretofore. Culture tubes of this fluid containing the five sulfonamide compounds previously described in saturated solution were inoculated with *E. histolytica*, incubated and examined at intervals. Growth occurred in all tubes and no greater inhibition was apparent than when cultures containing liver infusion agar were employed.

DISCUSSION

Although the results herein reported do not appear very encouraging, we feel that the sulfonamide compounds merit clinical trial in amebiasis and amebic dysentery. Such a trial becomes more important when one recalls the lack of correlation between the in vivo and in vitro activity of the sulfonamides in other conditions. Sulfanilamide appears to be the drug of choice, treatment perhaps being supplemented to advantage with retention enemas in which a high concentration of the compound may be employed. It may be noted that Saini obtained successful therapy with

sulfamethylthiazole in amebic dysentery in a child. The marked inhibition of *E. histolytica* in culture by the sulfonamide compounds, moreover, seems to indicate that synthesis of further compounds might prove of value.

Even though clinical trial may be unsuccessful, however, the use of *E. histolytica* seems to offer particular promise in the study of the action of the sulfonamides. This protozoon has the advantage that it is large, has definite structure and can be studied in the stained and living state.

SUMMARY

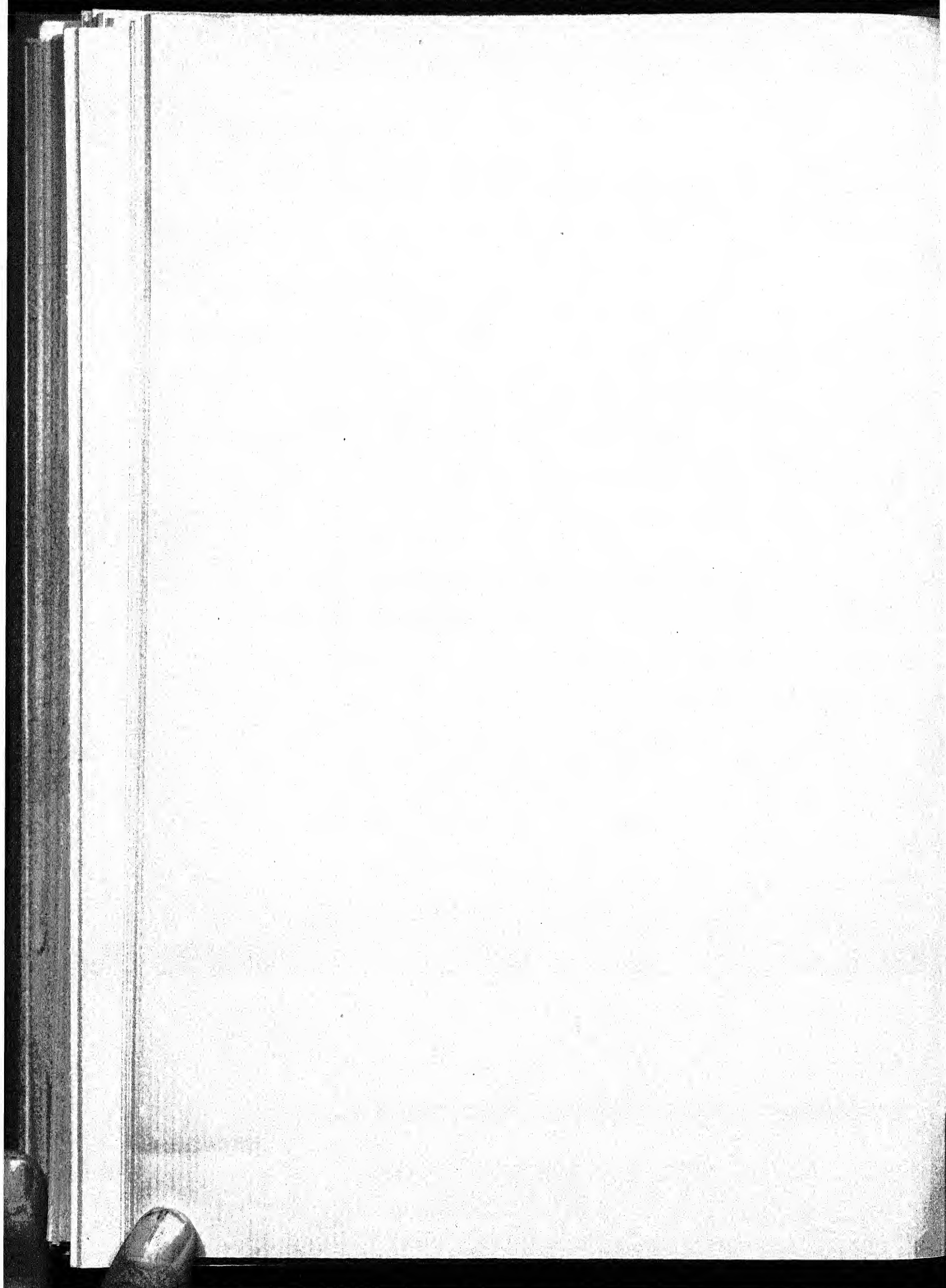
1. Five sulfonamide compounds, sulfanilamide, sulfathiazole, sulfapyridine, sulfaguanidine and sulfadiazine, all showed inhibitory action on the growth of *Endamoeba histolytica* in culture. Of these the action of sulfanilamide was the most pronounced.

2. At the same time these compounds often permitted persistence of living amebae in culture for longer periods of time than were obtained with media containing no drugs.

3. *Endamoeba histolytica* readily developed tolerance to the action of sulfanilamide.

BIBLIOGRAPHY

- BLACK, S., MCKIBBIN, J. M. AND ELVEHJEM, C. A. 1941 Use of sulfaguanidine in nutrition experiments. *Proc. Soc. Exper. Biol. and Med.* 47: 308-310.
- CLEVELAND, L. R. AND COLLIER, JANE 1930 Various improvements in the cultivation of *Entamoeba histolytica*. *Am. J. Hyg.* 12: 606-613.
- CRAIG, C. F. 1934 Amebiasis and Amebic Dysentery. Charles C. Thomas, Springfield, Ill.
- FAUST, E. C. 1941 Recent laboratory work throwing light on clinical amebiasis. *Rev. Gastroenterol.* 8: 197-203.
- SAINTI, M. 1940 I derivati metiltiazolici nell cura dell' amebiasi. *Rinasc. Med.* 17: 679.



NOTES ON NEMATODES FROM THE LUNGS AND FRONTAL SINUSES OF NEW YORK FUR-BEARERS

FRANS C. GOBLE AND ARTHUR H. COOK

Wildlife Research Center, N. Y. State Conservation Dept., Delmar, New York

Since the time of Redi scattered reports on the occurrence of various lung and sinus nematodes in fur animals have appeared, but strikingly little has been recorded which would indicate to what extent these infections are present in the wild. This article summarizes the parasitological findings in the respiratory tracts of the fur-bearers examined by us during the three years ending January 15, 1942.

Previous work on this group of hosts in this area has not been extensive. Hanson (1932) recorded lung and sinus worms from minks in northern New York and Stegeman (1939) noted skull lesions attributable to parasitism in skunks from the central part of the state. Goble and Cook (1941) observed the occurrence of lungworms in foxes in the Helderberg region and examinations of skunks in the vicinity of Albany led to the identification of the sinus worms and to the discovery of a new species of lungworm (Goble, 1942 a and b).

MATERIAL

All of the hosts examined were wild-trapped in New York State. The great majority of the specimens were taken in three counties bordering on the Hudson river within a radius of 50 miles of Albany. The remainder of the animals came from various other places in the state. The hosts examined are listed in Table 1 and the areas which were extensively collected may be seen in Map 1. The three counties in which more than two parasites were found were the ones in which the largest collections were made.

INCIDENCE

Table 1 indicates in a general way the extent of occurrence of respiratory parasites in the hosts examined. The percentages are presented only in order to convey the incidences in simple terms.

Data concerning relative infection with *Capillaria aerophila* in the two sexes was furnished by examination of a large series of red foxes from a limited region in southwestern Albany county and northern Greene county. In this area (the Helderberg plateau) a total of 68 males and 54 females was taken during the months of November and December in the years 1940 and 1941. The incidence of *C. aerophila* in males was 45.6% and in females 48.1%. It seems permissible, therefore, to com-

Received for publication, February 3, 1942.

bine the records for the two sexes in determining the incidence of this parasite. Incidences in three areas within this region ranged from 41 to 52% but the differences were not significant. *C. aerophila* occurred in 22.5% of the red foxes in Washington county but was not found in any of 25 animals from central New York.

The incidence of *Crenosoma vulpis* was significantly greater in males than in females (28% and 15.7%). Analysis of these data above gave

TABLE 1.—Occurrence of lung and sinus worms in fur-bearers in New York

Host	Number examined		Adults infected		Parasite
	Juvenile	Adult	Number	Per cent	
Opossum (<i>Didelphys virginiana</i>) .	8	14	0*		
Red fox (<i>Vulpes fulva</i>)	6†	184	39 66	21 36	<i>Crenosoma vulpis</i> (Dujardin, 1845) <i>Capillaria aerophila</i> (Creplin, 1839)
Gray fox (<i>Urocyon c. cinereoargenteus</i>)	1	88	15 6	17 7	<i>Crenosoma vulpis</i> <i>Capillaria aerophila</i>
Mink (<i>Mustela v. vison</i>)	0	44	22 4*	50 40	<i>Filaroides bronchialis</i> (Gmelin, 1790) <i>Skrjabinogylus nasicola</i> (Leuckart, 1842)
New York weasel (<i>Mustela frenata noveboracensis</i>)	15	15	6 4*	40 40	<i>Filaroides bronchialis</i> <i>Skrjabinogylus nasicola</i>
Bonaparte weasel (<i>Mustela c. cicognani</i>) .	0	5	0*		
Skunk (<i>Mephitis mephitis</i>)	6	57	11 18*	19 72	<i>Crenosoma zedleri</i> Goble, 1942 <i>Skrjabinogylus chiticoorum</i> Hill, 1939
Raccoon (<i>Procyon l. lotor</i>)	6	20	2	10	<i>Crenosoma</i> sp. (possibly <i>C. striatum</i> or <i>C. taiga</i>)
Beaver (<i>Castor canadensis</i>) . . .	5	4	0		
Muskrat (<i>Ondatra z. zibethica</i>) .	18	180	0		

* The frontal sinuses were examined in the following numbers of adults: 14 opossums, 10 minks, 10 N. Y. weasels, 5 Bonaparte weasels and 25 skunks. All juveniles of these species were examined for sinus worms and found negative.

† Animals designated as juveniles were all collected during the spring and summer and with the exception of one red fox, which was infected with *Crenosoma vulpis*, no lungworms were encountered in any of the young specimens.

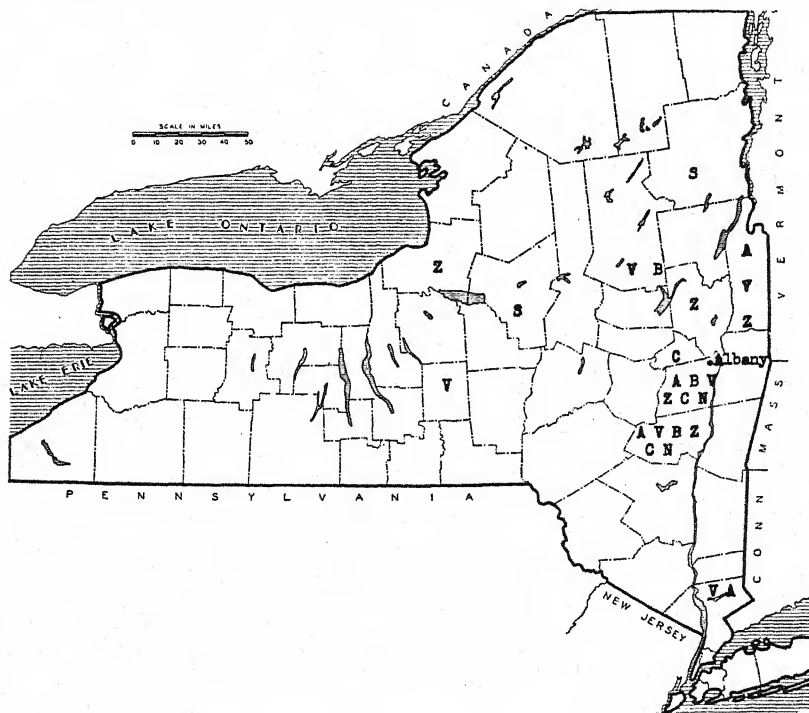
a χ^2 value of 5.43 which is significant with one degree of freedom. We are unable to offer any explanation for this difference at the present time. The incidence of *C. vulpis* was the same in red and in gray foxes and did not differ between the four counties where the largest fox collections were made.

PATHOLOGY

Due to the methods employed in killing trapped animals it is difficult to interpret accurately the significance of gross lesions in the lungs and the evaluation of the tissue changes attributable to the worms depends on

microscopic study of sectioned material. Two cases, however, which were uncomplicated by mechanical injury, are reported here.

In September, 1941, we received an immature female red fox, trapped at Piseco Lake, which was cachectic, poor in pelage and weak. Post-mortem examination revealed bilateral confluent bronchopneumonia and over 100 worms (*Crenosoma vulpis*) were exposed by cutting down the main bronchi. We estimate the total number of adult worms in the lung to have been over 300. Hanson (1933) reported 55 as the maximum



MAP 1. Known distribution of lung and sinus worms in New York fur-bearers. A, *Capillaria aerophila*; B, *Filaroides bronchialis*; C, *Skrjabinigylus chitwoodorum*; N, *Skrjabinigylus nasicola*; S, *Crenosoma* sp.; V, *Crenosoma vulpis*; Z, *Crenosoma sederi*.

infection with this parasite observed in ranch foxes. Another young red fox, trapped within 100 yards of the above on the same day and undoubtedly a litter mate, exhibited the same symptoms and died in captivity two days later but was not available to us for autopsy.

Early in January, 1942, an emaciated adult female skunk was brought to the laboratory. It was found dead near a highway and was suspected of having been killed by an automobile. Autopsy revealed that the cause of death was not traumatic. Examination of the head showed that an

abscess existed in the region of the frontal sinuses and that the bones dorsal and postero-ventral to the sinuses were eroded away so that the cranial cavity was continuous with the abscessed region which was exposed when the skin was removed from the top of the head. The porosity of the bone and the location of the lesions suggested that this condition might be the result of a serious sinus worm infection but no evidence of recent presence of worms could be found.

DISCUSSION

Although our examinations of the lungs and frontal sinuses of opossums disclosed no parasites, we have certain indications that both lung and sinus worms occur in these animals. We have in our collection a micro-slide preparation labelled "opossum lung," which was discarded from the histological collection of the University of Michigan Anatomy Department. This preparation shows nematodes in two bronchi, surrounded by mucous and cellular exudate, with accompanying peribronchitis. The date, locality and species of opossum are unknown. Weidman (1923) reported lung and sinus worms from opossums at the Philadelphia Zoo.

In the light of Hobmaier's (1941b) recent suggestion that foxes might sometimes be infected with the skunk lungworm, *Crenosoma mephitis*, it is interesting to observe that *Crenosoma vulpis* was the only species of that genus which occurred in foxes occupying the same range as the skunks which harbored *Crenosoma zederi*.

Disfigurement of the frontal region of the skull in various mustelids has been reported by a number of observers and previous to the description of *Skrjabinogylus chitwoodorum* Hill, 1939, these lesions were attributed to *S. nasicola* (often erroneously reported as *Filaroides mustelarum*). Recent identifications of sinus worms from five species of skunks (Hill, 1939; Hobmaier, 1941a; Goble, 1942a) seems to indicate that *S. chitwoodorum* is typical in the MEPHITINAE and that *S. nasicola* is the form commonly found in the MUSTELINAE (Hanson, 1932; Swales, 1938).

Swales found *Skrjabinogylus nasicola* in *Mustela cicognani anguinæ* and his records of skull lesions point to its presence in *M. arctica*, *frenata* and *longicauda* and to its absence in *M. vison*. We believe, however, the presence or absence of frontal dilatations is not a good index of the incidence of these parasites. We examined the sinuses of ten minks and ten weasels and in each species found infections in four of the ten. No external enlargement or abnormality was observed. On the other hand, no worms were found on opening the sinuses of a Bonaparte weasel which had an enlarged frontal region. The conditions under which alterations of the bone take place are obviously not well understood. Our Bonaparte weasels, which were negative, were taken in the same locality that yielded New York weasels with *S. nasicola* and *Filaroides bronchialis*.

Since muskrats belong to a new world genus and their presence in Europe is the consequence of introductions from this continent, the discovery by Schulz, Orlow and Kutass (1933) of the lungworm *Rodentocaulus ondatrae* in this host in the U.S.S.R. poses the question of whether this parasite was originally an inhabitant of muskrats in this hemisphere or whether it is a recent acquisition to the pulmonary fauna of *O. sibirica*. To our knowledge this nematode has not been found in North America.

SUMMARY

Observations on the incidence and identity of the parasites of the lungs and frontal sinuses of fur-bearers in New York State are recorded. These include: *Capillaria aerophila* and *Crenosoma vulpis* in red and gray foxes, *Skrjabinigylus nasicola* and *Filaroides bronchialis* in minks and New York weasels, *Skrjabinigylus chitwoodorum* and *Crenosoma zederi* in skunks and *Crenosoma* sp. in raccoons.

BIBLIOGRAPHY

- GOBLE, F. C. 1942a *Skrjabinigylus chitwoodorum* from the frontal sinuses of *Mephitis nigra* in New York. J. Mamm. 23: 96-97.
- 1942b *Crenosoma zederi* n. sp. (Nematoda: Metastrongyloidea) a new lungworm from the skunk (*Mephitis nigra*). J. Parasitol. 28: 381-384.
- GOBLE, F. C. AND COOK, A. H. 1941 Some lungworm records from foxes in New York. J. Mamm. 22: 456.
- HANSON, K. B. 1932 Parasites of minks and their control. Circular U. S. Dept. Agric. Bi-1235: 1-8.
- 1933 Tests of the efficacy of single treatments with tracheal brushes in the mechanical removal of lungworms from foxes. J. Am. Vet. Med. Assn. 82 (n.s. 35): 12-33.
- HILL, W. C. 1939 The nematode *Skrjabinigylus chitwoodorum* n. sp. from the skunk. J. Parasitol. 25: 475-478.
- HOBMAIER, M. 1941a Extramammalian phase of *Skrjabinigylus chitwoodorum* (Nematoda). J. Parasitol. 27: 237-239.
- 1941b Newer aspects of the lungworm (*Crenosoma*) in foxes. Am. J. Vet. Res. 2: 352-354.
- SCHULZ, R. E., ORLOW, I. W., AND KUTASS, A. J. 1933 Zur Systematik der Subfamilie Synthetocaulinae Skrj., 1932, nebst Beschreibung einiger neuer Gattungen und Arten. (Vorl. Mitt.) Zool. Anz. 102: 303-310.
- STEGEMAN, L. C. 1939 Some parasites and pathological conditions of the skunk (*Mephitis mephitis nigra*) in central New York. J. Mamm. 20: 493-496.
- SWALES, W. E. 1938 *Skrjabinigylus nasicola* (Leuckart, 1842) Petrow, 1927, a nematode parasitic in the frontal sinuses of American Mustelidae. Liv. Jub. Prof. Travassos, pp. 455-458.
- WEIDMAN, F. D. 1923 Parasites, in Fox: Disease in Captive Wild Animals and Birds; Philadelphia.

A STRAIN OF THE MOSQUITO *Aedes aegypti* SELECTED
FOR SUSCEPTIBILITY TO THE AVIAN MALARIA
PARASITE *Plasmodium lophurae*

WILLIAM TRAGER

Department of Animal and Plant Pathology, Rockefeller Institute for Medical
Research, Princeton, N. J.

When a group of mosquitoes, of a species known to be susceptible to a particular kind of malaria parasite, is allowed to feed on an infected host showing many gametocytes in its blood, some of the mosquitoes become very heavily infected, while others do not become infected at all. That this susceptibility or immunity is a hereditary character of the individual mosquito was conclusively shown by Huff (1927, 1929, 1931). By selective breeding he could in a few generations increase or decrease greatly the percentage of individuals of *Culex pipiens* susceptible to infection by *Plasmodium cathemerium*, depending upon whether the successive generations were reared from susceptible or immune mothers, respectively. The highly inbred lines of mosquitoes rapidly lost their fertility and a strain with higher susceptibility or immunity than the original stock could not be permanently established.

Experiments somewhat similar to those of Huff, but using *Aedes aegypti* and *Plasmodium lophurae*, have led to the establishment of a large colony of *A. aegypti* which for over a year has consistently proved to be at least twice as susceptible as the stock colony from which it was derived.

In his original description of *P. lophurae* Coggeshall (1938) reported that 9 out of 15 *A. aegypti* which fed on an infected chick showed oöcysts when they were dissected 6 days later. The mosquitoes were obtained from the colony maintained by me at the Rockefeller Institute in Princeton. Later work by Coggeshall (1941a), Huff (1940), and myself with the same strain of *Aedes aegypti* has indicated that the percentage of infection is usually much less than 60%. Huff (1940) found that the parasites did not develop at all in *A. aegypti* at temperatures less than 28° C, a fact which had been independently observed early in the course of the present work. But even if the mosquitoes were kept at 30° C the percentage of infected individuals obtained from groups fed on chicks showing many gametocytes was always low. Much better results were obtained by Laird (1941) with the closely related mosquito *A. albopictus* which proved to be about 90% susceptible to *P. lophurae*. *Culex restuans* and *Aedes atropalpus* also were highly susceptible and showed sporozoites

in the salivary glands (Laird, 1941). Interestingly enough, *P. lophurae* produced fairly heavy stomach infections in *Anopheles quadrimaculatus* (Coggeshall, 1941a), but in only one mosquito have sporozoites thus far been found (Hurlbut and Hewitt, 1941). Of 167 *C. pipiens* dissected, 1.0% showed infection by *P. lophurae* (Coggeshall, 1941a).

METHODS

A strain of *P. lophurae* obtained from Coggeshall and maintained by intracerebral passage in baby chicks (Trager, 1941) was used, except in one experiment in which use was made of a sub-strain maintained in ducks (Wolfson, 1940). Mosquitoes were allowed to feed only on heavily infected chicks which showed abundant exflagellation and, in a stained smear, usually at least one gametocyte per oil-immersion field of the microscope.

The stock colony of *A. aegypti* has been bred continuously in this laboratory for 8 years. The adults are kept in a large cage supplied with a cotton pad moistened with water and another pad moistened with sugar solution and bearing a few crushed raisins. They are given a blood meal on a guinea pig twice a week. Eggs are laid on filter paper half immersed in a beaker of water. They are not used for breeding purposes until at least a week old. The larvae are reared in tap water to which suitable small amounts of dry powdered yeast and powdered milk are added daily.

The susceptible strain "D," the establishment of which will be described presently, is now maintained in the same manner.

A stock colony of *Aedes albopictus*, derived from eggs which were very kindly sent me by Dr. G. L. Orth of the Army Medical School and Dr. R. L. Laird at Johns Hopkins, is now kept going at a high level by the same methods used for *A. aegypti*.

For the selection experiments, a group of mosquitoes was allowed to feed on a suitable chick. A number of the engorged females were then isolated in half-pint milk bottles, 1 ♀ per bottle. Each bottle contained water to a depth of 2 cm and a circle of 12.5 cm filter paper inserted vertically against the side of the bottle. This filter paper provided a foothold for the mosquito as well as a place on which it could lay its eggs. The bottle was covered with a piece of gauze having in its center a small cotton-plugged hole, through which the mosquito was introduced from the catching tube. A pledget of cotton soaked in sugar solution was placed on top of the gauze. This was moistened daily. Occasionally, a small amount of water had to be added to the bottle to replace loss by evaporation. Female *A. aegypti* isolated under these conditions laid eggs and usually survived the 10-14 days required for the experiments. Since *A. aegypti* eggs, if kept slightly moist, survive but do not hatch until immersed in water, the eggs were left with the parent female until the

latter had been dissected. On the basis of the dissection results, suitable batches of eggs were selected for further breeding.

The stock and experimental mosquitoes were all kept in a greenhouse at temperatures of 30–32° C (usually 30–31° C).

RESULTS

The selection experiments.—In Table 1 are indicated results with all the selections which led to the establishment of strain D, as well as results with the offspring of other related females. A female was considered infected if it showed either oöcysts or sporozoites. The third

TABLE 1.—Results of selective breeding for susceptibility, starting with stock *Aedes aegypti* females

Generation	Group No.	Derived from infected ♀ no.— of group no.—*	Number dissected	Number infected	Per cent infected	Number with oöcysts only
I	1	...	27	3	11	2
II	2†	1:1	20	18	90	3
	3	2:1	13	7	54	4
	4	3:1	10	3	30	1
III	5†	1:2	10	6	60	0
	6	2:2	7	4	57	1
	7	3:2	11	4	37	1
	8	4,5,6:2	17	8	47	3
IV	9†	1:5	15	12	80	4
	10	2:5	20	15	75	5
	11	1,2,3:8	12	5	42	0
V	12	1-8:9 and 1-6:10	28	4	14	0
	13	9:9	6	2	33	0
	14†	10:9	7	5	71	0
	15	11:9	4	3	75	0
	16†	12:9	9	8	90	0
	17	7:10	11	7	63	0
VI	18†	1:14	7	4	57	..
	19	1:15	8	7	88	..
	20	1:16	3	3	100	..
	21†	2:16	12	6	50	..
VII	22†	1:18 and 1:21	3	1	33	..
	23	1:19	9	6	67	..
	24	1:20	4	3	75	..
VIII	25	1:23	8	5	62	..
	26	2:23	2	1	50	..
	27	1:24	4	1	25	..
IX	28	1,2:25 and 1:26 and 1:27	13	12	92	0
VIII	29†	Unselected— from 22	13	12	92	0
X	30	Unselected— from 28	19	17	90	0

* The number or numbers to the left of the colon refer to the female or females of the group number given to the right of the colon.

† Indicates the selections which led eventually to strain D.

column of the table gives the source of the parent female (or females) of each group of mosquitoes tested for susceptibility. It will be noted from Table 1 that with the first selected generation (generation II) there was already a marked increase in the percentage of susceptible individuals and further selection did not produce any appreciable further increase. This result is very similar to those obtained by Huff (1929) with *C. pipiens* and *P. cathemerium*; by Storey (1932) with the leafhopper *Cicadulina mbila*

and the virus, streak disease of maize; and by Black (1941, 1943) with the leafhopper *Aceratagallia sanguinolenta* and the virus, potato yellow-dwarf. However, if unselected eggs were reared from the earlier generations in the course of the selections, the resulting adults had about the same susceptibility as the original stock, whereas when unselected eggs were reared after VII or IX generations (groups 29, 30 of Table 1) the resulting adults were highly susceptible. It was found in this study, just as Huff had found with *C. pipiens*, that the fertility of the mosquitoes appeared to diminish with continued inbreeding. By generation VI, the fifth selected generation, the eggs laid by several of the engorged, infected females (in a line not shown in Table 1) failed to hatch; and by generation VII there were engorged females which laid no eggs. Many of the viable batches which were laid were very small. The females of group 28 laid fairly numerous eggs, all of which were combined to give group 30 with a total of only about 60 or 70 adults. The females of group 30 were given blood meals on June 18, 21, and 24, 1940. They did not begin to lay eggs until June 23, although the stock *A. aegypti* regularly begin laying eggs $2\frac{1}{2}$ –3 days after their blood meal. Although a fair number of eggs was finally obtained, rather few of them hatched. Enough were reared, however, to give another generation of adults which again laid some eggs. But from these eggs only 2 females and a few males could be reared. One of the females was too weak to feed. The other one took two meals of human blood and laid a small batch of eggs 7 days after the first meal. None of these eggs hatched. Thus 8 generations of inbreeding reduced the fertility to such a point that further mixed breeding could not restore it.

In group 22 the females were kept as long as possible in order to secure a maximum number of eggs. The 3 which were dissected represent only the 3 last survivors, so that the unselected eggs used to give group 29 came from considerably more individuals than these 3. Since the fertility of this line also was obviously going down, selective breeding was discontinued. Eggs from group 29 gave only 12 females which took blood meals. By nursing these along, enough eggs were collected for the establishment of a small colony which was treated just like a stock colony (with freshly reared offspring added once in 10 days) for 6 weeks. Then a batch of females was fed on an infected chick. Out of 29 dissected, 18 (62%) were infected. Eggs from 14 of these infected females were reared, and the adults so obtained (on October 11, 1940) were used to establish a new colony, now designated as strain D. No further selection has since been exercised.

Tests of strain D.—From time to time small groups of females from this colony have been fed on chicks infected with *P. lophurae*, and usually about 40–60% have become infected. In two tests, with chicks which

showed fairly active exflagellation and about 1 gametocyte per field, a considerably lower infection rate was obtained, but in both cases it was higher than in comparable groups of stock *A. aegypti* fed on the same chick at the same time. In one of these experiments none out of 20 stock *A. aegypti* and 4 out of 22 strain D *A. aegypti* were infected. In the other experiment, 1 out of 24 stock *A. aegypti*, 4 out of 22 strain D *A. aegypti*, and 5 out of 16 *A. albopictus* were infected. That the low infection rate obtained in these experiments may have been the result of gametocytes which were insufficient either in numbers or in infectivity is indicated by the unusually low percentage of infected *A. albopictus* obtained in the latter experiment. These were fed on the same chick used in this experiment for the two strains of *A. aegypti*.

The most recent test, conducted in the early part of December 1941 with comparable groups of the two *A. aegypti* strains and of *A. albopictus*, deserves description in detail. Since the larval stage of *A. albopictus* takes a few days longer than that of *A. aegypti*, the eggs of this species intended for the experiment were hatched several days sooner. In this way, the adults of all 3 groups emerged within 3-4 days of each other. They were about 4-7 days old when they were allowed to feed on a duckling which was 4 weeks old and had been infected 5 days previously. From each group, 200 engorged females were isolated. Fifty from each group were dissected 10 days later, and both the stomachs and salivary glands examined. The results are summarized in Table 2. It is obvious

TABLE 2

Mosquitoes used	Number out of 50 which showed						Per cent infected with sporozoites
	Sporozoites	1-5 oöcysts*	5-20 oöcysts	More than 20 oöcysts	Oöcysts but no sporozoites	Oöcysts with black spores	
<i>A. aegypti</i> —Stock ..	15	12	7	3	7	11	30
<i>A. aegypti</i> —Strain D	31	17	15	2	3	28	62
<i>A. albopictus</i>	43	10	12	21	0	0	86

* In all cases, the oöcyst figures include all oöcysts found regardless of size and regardless of whether they contained black spores.

that strain D, after more than a year of nonselective breeding, has retained its character of being considerably more susceptible to infection with *P. lophurae* than the stock from which it was derived. It is less susceptible than *A. albopictus*. Half of the infected *A. albopictus* had more than 20 oöcysts on the stomach wall, while only 2 strain D and 3 stock *A. aegypti* had more than 20. Of the total numbers which showed oöcysts, more of strain D had 5-20 per stomach than of the stock. While the stock

showed 7 abortive infections (oöcysts but no sporozoites), strain D showed only 3 and *A. albopictus* none. It is also of interest that no oöcysts containing black spores were noted in the *A. albopictus* group, whereas the majority of the infected individuals in both other groups had such oöcysts. These results suggest that the mechanism of resistance may be effective not only against the zygotes, as shown by Huff (1934), but also against oöcysts in later stages of development. Very heavy salivary gland infections were shown by 10 stock *A. aegypti*, 20 strain D *A. aegypti* and 36 *A. albopictus*. The salivary glands of these heavily infected individuals had a characteristic fragility and stickiness lacking in uninfected or lightly infected glands, and they could be readily recognized in the course of dissection under a low power dissecting microscope. This has been previously noted by Giovannola (1933) in heavily infected salivary glands of *Anopheles*.

The infection of chicks by sporozoites of P. lophurae.—This has been accomplished repeatedly, using 1- to 2-day-old chicks and sporozoites from either *A. aegypti* or *A. albopictus*. In one experiment a chick fed on by 4 infected mosquitoes showed parasites 15, but not 11, days later. Another chick inoculated intracerebrally with the teased up glands of the same 4 mosquitoes first showed infection at the same time. A chick inoculated intracerebrally with material from one infected *albopictus* showed parasites after 23 but not after 19 days.

Laird (1941) had difficulty in infecting ducklings with sporozoites of *P. lophurae* either by bite or by inoculation of infected *A. albopictus*. He did obtain transmission by both methods, but the infections which resulted were much lighter than those ordinarily obtained by blood inoculation. This fact is considered by Laird and by Wolfson (1941) to indicate that *A. albopictus* may not be a suitable vector for *P. lophurae*. In the present experiments, the infections obtained by sporozoite inoculation were also very light. But before drawing any conclusions it is important to consider the following facts. In routine blood inoculation of ducklings, millions or billions of parasites, suspended in their natural medium (blood) are introduced intravenously. In Laird's experiments with sporozoites, unknown and probably very much smaller numbers of parasites were introduced either into capillaries (by mosquito bite), or intramuscularly (by injection). It has been shown that at least 200 asexual parasites must be inoculated intracerebrally into a 1- to 2-day-old chick to produce an infection apparent 11 days after (Trager, 1941). When the glands of one or a few mosquitoes are enormously diluted with saline, it is altogether likely that rather small numbers of viable sporozoites are actually injected. Hence it is not surprising that chicks inoculated intracerebrally with such material take about 10-15 days to show blood infection. The light infections thus far obtained with sporozoite inoculation

may well be due to the fact that *P. lophurae* in general produces heavy infections only if very large numbers of parasites are injected. It is also pertinent to note that while one asexual parasite of *P. cathemerium* can infect a canary (Stauber, 1939), at least 200 sporozoites of the same species were required to produce infection (Rozeboom and Shah, 1934).

DISCUSSION

The experiments here reported confirm those of Huff (1929, 1930, 1931, 1934), in showing once again, with a different genus of mosquito and a different species of *Plasmodium*, that the individual susceptibility or resistance of a mosquito to infection by malaria parasites is a heritable characteristic. In addition, it has now been possible to establish a "susceptible" strain and to maintain it in large numbers for comparison with the original more resistant stock from which it was derived. Huff concluded (1934) that his data indicated that susceptibility of *Culex pipiens* to *P. cathemerium* behaved like a simple recessive Mendelian character. The data for *Aedes aegypti* do not support such a conclusion. In both cases, a precise genetic analysis is difficult because there is no direct way of knowing the susceptibility of the males. Such a genetic analysis has however been possible with 2 species of leafhoppers which transmit 2 different plant viruses. Here both males and females could be tested for ability to transmit. Storey (1932) developed by selection two races of *Cicadulina mbila*, one able to transmit streak disease of maize and the other unable. The ability to transmit behaved as a simple sex-linked dominant Mendelian character. Black (1941, 1943) similarly obtained 2 races of *Aceratagallia sanguinolenta*, able and unable respectively to transmit potato yellow-dwarf. However, hoppers able to transmit the virus appeared in colonies of the inactive race, even though all their ancestors for 10 generations had failed to transmit. The reverse situation was also obtained, suggesting that ability to transmit is controlled by multiple characters.

Naturally occurring or artificially produced strains of mosquitoes, differentiated on the basis of characters other than susceptibility, have in several instances been tested for their susceptibility to malaria parasites. Tate and Vincent (1934) found no difference in susceptibility to *P. relictum* of an anautogenous English and autogenous Greek, Hungarian, Maltese and cross-bred strains of *Culex pipiens*. *Anopheles elutus* and *A. maculipennis* were equally susceptible to infection with *P. vivax* and *P. falciparum*, the greater importance of *elutus* in the transmission of malaria being the result of its greater preference for human blood (Barber and Rice, 1935). A strain of *A. quadrimaculatus* from Tennessee had the same degree of susceptibility to both *P. falciparum* and *P. vivax* as another strain from Florida (Boyd, 1941). Two strains of

A. quadrimaculatus produced by selection on the basis of larval markings were almost equally susceptible to infection with the monkey parasite *P. cynomolgi* (Coggeshall, 1941b). Thus, so far strains of the same species of mosquito differing in susceptibility to malaria parasites have been obtained only by selection for this particular character.

SUMMARY

By selective breeding from infected females, a strain of *Aedes aegypti* was produced which, after more than a year of mass, nonselective breeding, has maintained its character of being much more susceptible to infection with *Plasmodium lophurae* than the stock from which it was derived.

BIBLIOGRAPHY

- BARBER, M. A. AND RICE, J. B. 1935 Malaria studies in Greece. The malaria infection rate in nature and in the laboratory of certain species of *Anopheles* of East Macedonia. *Ann. Trop. Med. and Parasitol.* 29: 329-348.
- BLACK, L. M. 1941 Hereditary variation in the ability of the clover leaf hopper to transmit potato yellow-dwarf virus. (Abstract.) *Phytopath.* 31: 3.
- 1943 Genetic variation in the clover leafhopper's ability to transmit potato yellow-dwarf virus. (In press.)
- BOYD, M. F. 1941 The comparative susceptibility of two strains of *Anopheles quadrimaculatus* to infection with human malaria parasites. *Am. J. Trop. Med.* 21: 751-753.
- COGGESHALL, L. T. 1938 *Plasmodium lophurae*, a new species of malaria parasite pathogenic for the domestic fowl. *Am. J. Hyg.* 27: 615-618.
- 1941a Infection of *Anopheles quadrimaculatus* with *Plasmodium cynomolgi*, a monkey malaria parasite, and with *Plasmodium lophurae*, an avian malaria parasite. *Am. J. Trop. Med.* 21: 525-530.
- 1941b Strains of *Anopheles quadrimaculatus*. Inheritance of color patterns in the larvae of *Anopheles quadrimaculatus*. *Am. J. Trop. Med.* 21: 755-765.
- GIOVANNOLA, A. 1933 Caratteristiche alterazioni della glandole salivary dell' *Anopheles maculipennis* infetto da parassiti malarigeni. *Riv. Malariol.* 12: 1140-1146.
- HUFF, C. G. 1927 Studies on the infectivity of plasmodia of birds for mosquitoes, with special reference to the problem of immunity in the mosquito. *Am. J. Hyg.* 7: 706-734.
- 1929 The effects of selection upon the susceptibility to bird malaria in *Culex pipiens* Linn. *Ann. Trop. Med. and Parasitol.* 23: 427-440.
- 1930 Individual immunity and susceptibility of *Culex pipiens* to various species of bird malaria as studied by means of double infectious feedings. *Am. J. Hyg.* 12: 424-440.
- 1931 The inheritance of natural immunity to *Plasmodium cathemerium* in two species of *Culex*. *J. Prev. Med.* 5: 249-259.
- 1934 Comparative studies on susceptible and insusceptible *Culex pipiens* in relation to infections with *Plasmodium cathemerium* and *P. relictum*. *Am. J. Hyg.* 19: 123-147.
- 1940 Quantitative studies on size, variability and growth rates of oöcysts of different strains of avian malaria. *Am. J. Hyg.* 32 C: 71-80.
- HURLBUT, H. S. AND HEWITT, R. 1941 Sporozoites of *Plasmodium lophurae*, an avian malaria parasite, in *Anopheles quadrimaculatus*. *Pub. Health Rep., U. S. Pub. Health Service* 56: 1336-1337.

- LAIRD, R. L. 1941 Observations on mosquito transmission of *Plasmodium lophurae*. Am. J. Hyg. 34 C: 163-167.
- ROZEBOOM, L. AND SHAH, K. 1934 Preliminary studies on the production of bird malaria infections by the injection of sporozoites. J. Parasitol. 20: 198.
- STAUBER, L. A. 1939 Factors influencing the asexual periodicity of avian malarias. J. Parasitol. 25: 95-116.
- STOREY, H. H. 1932 The inheritance by an insect vector of the ability to transmit a plant virus. Proc. Roy. Soc. 112 B: 46-60.
- TATE, P. AND VINCENT, M. 1934 The susceptibility of autogenous and anautogenous races of *Culex pipiens* to infection with avian malaria (*Plasmodium relictum*). Parasitol. 26: 512-522.
- TRAGER, W. 1941 Studies on conditions affecting the survival in vitro of a malarial parasite (*Plasmodium lophurae*). J. Exper. Med. 74: 441-462.
- WOLFSON, F. 1940 Successful cultivation of avian plasmodia in duck embryos. Am. J. Hyg. 32 C: 60-61.
- 1941 Avian hosts for malaria research. Quart. Rev. Biol. 16: 462-473.

OBSERVATIONS ON THE LIFE HISTORY AND TAXONOMIC
RELATIONSHIPS OF THE TREMATODE
*ASPIDOGASTER CONCHICOLA**

CHARLES O. WILLIAMS

In 1827, von Baer described a trematode which he named *Aspidogaster conchicola*. The original material was from a fresh-water mussel of the genus *Anodonta*, from Prussia. This same species has long been known to occur in the pericardial and renal cavities of North American UNIONIDAE, but most of the studies carried on by American investigators have dealt chiefly with morphology and distribution of the species. Kelly (1899) carried on extensive studies of the host relationships of this species in the Illinois River and Stunkard (1917) made a study of the general morphology which confirms and adds somewhat to the earlier observations by Stafford (1896). There has been but fragmentary information concerning the development of this parasite of the UNIONIDAE. Stafford (1896), Faust and Tang (1936), and others have made the suggestion that the life cycle is fundamentally direct, i.e., that the young worm develops directly into an adult, either in the same host individual which shelters the mature worm, or in another individual of the same or of a different species. The systematic position of *Aspidogaster* has long been in doubt. Whether it belongs in MONOGENEA, DIGENEA, or an intermediate group has been a controversial question. It is the object of this paper to trace the life cycle of this worm and to review critically the evidence as to its systematic position.

In the present study, the process of hatching of the eggs of *Aspidogaster* has been observed and, in natural infections, series of immature stages have been found representing fairly complete developmental stages all within the same host species. *Aspidogaster conchicola* therefore differs from the general pattern of host-parasite relationship among trematodes in that the entire life cycle occurs within an invertebrate host.

UNIONIDAE from the Sangamon River near Mahomet, Illinois; from the Salt Fork, at Homer Park, Illinois; and from the Wabash River at West York, Illinois, are the hosts of the *Aspidogaster* used in this study. A detailed list of the host species will be presented in another paper dealing more directly with relationships to the host.

Received for publication, February 8, 1942.

* A contribution from the Zoological Laboratory of the University of Illinois, Urbana. These results, which were in preliminary draft when Ensign Williams was called into the armed services, have been edited by H. J. Van Cleave, under whose direction the research was conducted.

MORPHOLOGY OF ADULT

The elongated body of *Aspidogaster conchicola* is almost entirely covered on the ventral side by a large, alveolated sucker. The body of the worm, exclusive of the ventral sucker, is typically spindle shaped and during locomotion may be stretched to twice its normal length. The highly distensible mouth, located at the end of the neck, is surrounded by an oral sucker. The pharynx, posterior to the oral sucker, is small, but in the living worm it may be seen opening and closing. The intestine, a blind sac which extends almost the entire length of the body, is unbranched but broadens slightly as it continues posteriorly.

The genital pore, located in a slight depression at the union of the ventral sucker and the neck, is on the ventral surface of the neck and at the anterior end of the ventral sucker. A common genital atrium receives both the cirrus, within the cirrus sac, and the uterus. The small ovary is located in the ventral portion of the body. It lies slightly dorsal to the ventral sucker, about midway the length of that organ. The oviduct, leaving the ovary, continues toward the posterior end of the body. It is joined by the Laurer's canal, the paired vitelline canals, and the Mehlis' gland. When filled with eggs, the uterus is tightly coiled back and forth. Upon reaching the posterior end of the body it curves forward toward the anterior end to reach the genital atrium. The unpaired testis, somewhat larger than the ovary, lies along the ventral surface of the body, dorsal to the ventral sucker and posterior to the ovary. The vas deferens leaves the testis posteriorly, then turns anteriorly and continues until it reaches the cirrus sac. Here it empties into the widened seminal vesicle, which ends in the cirrus. The very numerous vitelline follicles are placed laterally in the body. They extend from the posterior end of the cirrus sac to a point just about even with the posterior end of the testis.

The excretory system is one of the most highly developed systems present in the adult *Aspidogaster*. A common excretory pore is present at the posterior end of the worm. This pore receives waste products from two individual excretory bladders whose ducts unite only a short distance before the excretory pore is reached. A single excretory canal empties into each bladder. These canals continue anteriorly and branch out to receive waste products from all parts of the body. Faust (1922: 114) states that there is a total of 243 capillaries and flame-cells for each side of the body of *Aspidogaster conchicola*.

LARVAL DEVELOPMENT

By gradual transformations the larva of *Aspidogaster* acquires the adult stage without sharp metamorphosis to mark off critical, fixed forms. A series of arbitrarily selected steps in the development are described below. The necessity for following this procedure instead of the practice

of describing distinct larval stages is imposed by the fact that larval development is direct.

In observing living adults of *Aspidogaster conchicola*, some of the uterine eggs may be observed to contain active embryos. Faust (1922, Plate XIV) has figured the hatching from the egg membranes of one of these, but the full development has not been traced previously.

First Larval Stage.—Upon the emergence from the egg membranes, the larva (Fig. 1) is not a ciliated miracidium but is a young worm with rudiments of most of the adult structures already discernible. The operculate, embryonated egg is 128 to 134 μ long and 48 to 50 μ wide. Upon hatching, the larva is 130 to 150 μ long and 50 to 55 μ in diameter. The body of the newly-hatched larva as viewed laterally, is generally seen to be divided into three main divisions: anterior, middle, and posterior, each of which contains a primordium of some later structure. These divisions are not separated by actual walls or membranes, but are set off by external grooves.

At the anterior end of the newly hatched larva (first stage) a relatively large oral sucker is recognizable. The oral sucker and the pharynx, extending posteriorly from it, are lined with contractile fibers, for the opening and closing of the sucker and pharynx are common occurrences in newly hatched individuals.

The larval intestine is a cylindrical cavity located in the central division of the body. As a general rule, it is smaller in diameter than the oral sucker. The intestine, together with the excretory bladders which lie on each side of it, at times cause the middle portion of the body to become distended.

In the posterior division of the larva (Fig. 1 VS), the ventral sucker appears as an internal pocket or sac-like organ. In living larvae it contracts and expands. This pocket opens to the ventral surface of the body by a deep cleft which lies just anterior to the small tail-like projection. Surrounding the external opening are muscles which enable the larva to open or close the suckers. At this early stage of development the sub-terminal ventral sucker is capable of becoming attached to objects. Two newly hatched larvae under observation clung together by means of their ventral suckers for about 48 hours. The depth and width of the ventral sucker could not be obtained accurately in living specimens. The larvae were too active for camera lucida drawings, so it became necessary to fix the worms. Alcohol-formol-acetic (85 parts of 85% alcohol, 10 parts formalin, 5 parts glacial acetic) proved a desirable killing agent. However, it was found that with all killing agents the pocket of the ventral sucker was somewhat distorted.

The locomotor ability of newly hatched larvae is negligible. No swimming movements have been noticed. In fact, the suckers of the

newly hatched worm are immediately functional as organs of fixation. After several minutes of activity, larvae under observation have shifted their positions only a few microns. The conclusion may then be reached that either the larvae remain within the viscera of the same host individual throughout their entire life cycle, from egg stage to the adult, or they are transferred passively by water current from host to host.

Second Larval Stage.—Of the numerous immature individuals that have been observed, a second stage larva (Fig. 2) has been recognized, characterized by (1) an increase in size from 150 μ in length to 275 μ ; (2) loss of body constrictions; and (3) attainment of greater powers of locomotion. In this stage there has not yet appeared any conspicuous change in the subterminal ventral sucker.

An adult *Aspidogaster* taken from the pericardial cavity of a mussel, *Anodonta grandis*, had a larva of the second stage, 272 μ long and 48 μ wide, clinging to the external lip of the oral sucker. When the larva was detached from the adult and placed on a slide, it moved like a leech, shifting the oral and ventral suckers. Even though this larva was only about 100 μ longer than the newly hatched larva, its power of locomotion was pronounced, though it lacked ability to swim.

The pharynx and intestine of this larva were more highly developed than those of the newly hatched larvae. The ventral sucker was 64 μ in diameter, as compared with that of the recently hatched larva which was 40 μ , and the oral sucker was 68 μ in diameter.

Third Larval Stage.—A third larva taken from the pericardial cavity of a mussel heavily infected with adults, was 880 μ long and about 180 μ wide. The oral sucker, when fully extended and attached to the substratum of the container, was approximately 195 μ in diameter.

The shape of this larva is greatly modified from that of the previously described forms. Instead of the characteristic shape of newly hatched larvae in which the body seems divided into three vague divisions, this later form has lost its constrictions. The oral sucker, more prominent than that of the younger larvae, is located at the end of a long neck-like structure, much as in the adult worm, though the ventral sucker has not yet attained the form and position characteristic of the mature worm.

Posterior to the oral sucker and situated in the neck, is the pharynx, in shape identical with the pharynx of the adult. Just posterior to the pharynx is the sac-like intestine, which extends approximately three-fourths of the body length. In sagittal sections, masses of primordial germ cells were observed in this stage (Fig. 9).

Although the general body shape of this third larval stage is different from that of the newly hatched larvae, there are points of recognizable resemblance. In the first place, the positions of the oral sucker, ventral sucker, intestine, and rudimentary tail are much the same. In the very

young larvae, the ventral sucker is located in the posterior end of the worm, with the rudimentary tail just posterior to it (Figs. 6 and 7). This same arrangement is found in the later larval forms. The ventral sucker of the third larval stage (Fig. 10) bears only a slight resemblance to that of the adult worm. It has assumed larger proportions in this later larval form, for almost the entire posterior fourth of the worm is filled by the large sac-like sucker (Fig. 8) which extends ventrally from the dorsal level of the intestine, opening on the ventral surface (Figs. 6 and 7). The lips of this sucker have a muscular rim and do not spread out over any of the surrounding tissue. As the larva develops, this pocket gradually becomes more shallow and the lips of the pocket begin to extend both anteriorly and posteriorly over the ventral surface of the worm. The greater extension of this outgrowth of the lips is anterior in direction. The body wall seems to have grown down over the increased ventral sucker, for in this stage when the worm is attached by the ventral sucker, the body may be lifted free from the object to which it is attached, being entirely supported by the ventral sucker. In recently hatched larvae (Fig. 1), the entire ventral surface of the worm is in contact with the floor of the containing vessel. In the first and second larval stages, only a circular ring of muscles is seen as the external part of the sucker. The third stage represents a transition toward conditions in the adult worm, where a foot-like ventral sucker has no opening but is made up of numerous alveoli spread over the ventral surface of the body.

Fourth Larval Stage.—A fourth and more advanced larval stage (Fig. 3) was found in the pericardial cavity of a heavily infected mussel. Body shape and structure, as well as the use of external parts, were almost identical with those of the adult. The larva was from 1200 to 1400 μ long when extended and about 320 μ wide. The ventral sucker was about 560 μ long and 320 μ wide. The oral sucker was larger and a little more prominent than in the preceding larval stage just described. It was no longer of major importance in locomotion.

The ventral sucker was in the last stages of metamorphosing from an invaginated pocket-like sucker, of which the greater part was contained inside the body, to the ventral sucker external on the body of the adult (Fig. 4). The position and general outline of the ventral sucker of this larva were identical with those of the adult but the alveoli were not yet fully formed. As the evagination of the ventral sucker continued, the portion spread out over the ventral body surface began to take on the general characteristics of the adult structure. In some individuals of the fourth stage larva, the two median rows of alveoli were not yet formed, but the boundaries of the lateral rows were distinct. Near the posterior end of the ventral sucker, a slit-like opening of a shallow pit was recognized (Fig. 5) as a vestige of the orifice of the deep internal sucking cup

of the earlier larval stages. The opening of the pit did not extend completely across the sucker laterally, but stopped short of the margin of the sucker on both sides. The slit-like opening of a living worm was in the shape of a quarter-moon. The boundaries of the lateral rows of alveoli could be seen extending up to the rim of the pit, then extending very faintly across the bottom of the depression, and finally reappearing on the other side. The posterior rim of the pit did not extend to the posterior end of the sucker, but stopped just short of it. At times the worm would curl the posterior tip of the sucker forward, in this manner deepening and enlarging the pit (Fig. 9). This was noticed especially whenever the worm had lost its anchorage and was securing a new attachment. Crawling in this larva was at a relatively much slower rate than in the previously described stage. The oral sucker no longer assisted in locomotion, except whenever the ventral sucker became detached. Then the oral sucker would fasten itself until the ventral sucker could regain its attachment.

The excretory system of this larva was well developed. Numerous flame cells were observed and the main branches as seen in the adult were present. The large loops of the excretory tubules in the region of the pharynx were particularly evident. These extended posteriorly, one on each side of the body in the region of the attachment of the ventral sucker to the body proper, where each ended in an excretory bladder. These bladders were separated by a much longer partition than are the bladders of the adult. The bladders were connected to the exterior by a very short common canal. At times, muscles in this region contracted

TABLE 1.—*Comparison of various stages in the development of Aspidogaster conchicola. All measurements of living, active worms are expressed in microns*

Stage	Dimensions		Oral sucker diameter	Ventral sucker diameter	Description of ventral sucker
	Length	Diameter			
Adult	2500 to 2750	1150 to 1225	290	554	Composed of four rows of rectangular alveoli. Covers ventral surface.
Embryonated egg	128 to 134	48 to 50			
Stage I	130 to 150	50 to 55	44	40	Deep sac-like organ near posterior end of larva.
Stage II	275	50	68	64	Deep sac-like organ.
Stage III	880 to 960	175 to 180	195	205	Sac-like organ beginning to evaginate. Not as deep as in Stage II.
Stage IV	1200 to 1440	320	275	320	Lateral alveoli appear, median not yet fully formed; only shallow pit remains of sac-like ventral sucker.

and caused a portion of the external body wall to be slightly invaginated. During these periods of contraction the external pore was greatly enlarged and the connections of the bladders with it were visible. After discharge, the bladders were smaller but as excretory products poured in, the bladders again became distended. This distention continued for a time, and then the waste products were forced out through the external pore. The smaller excretory tubules were ciliated and structurally seemed to be identical with those of the adult. Inside the bladders were crystal-like particles, slightly brownish in color, which shifted positions every time the bladders contracted. These particles were not connected to the walls of the bladders, but they were not forced out through the excretory pore.

SYSTEMATIC POSITION OF *Aspidogaster*

The systematic position of the group to which *Aspidogaster conchicola* belongs has been in doubt for many years. The genus has been considered by some workers as belonging to the MONOGENEA; by others, to the DIGENEA (e.g., Ward, 1918); and yet by other investigators (Faust and Tang, 1936: 498-499), as representing a group intermediate in position between the MONOGENEA and the DIGENEA. These worms are in all probability not MONOGENEA, for they lack posterior sucking discs and chitinous hooks or anchors; the excretory pore is single and posterior in position rather than paired anteriorly; and their digestive tract is always rhabdocoele in type. At the same time, there is no evidence that they have an alternation of generations, a prerequisite for typical DIGENEA. Inside the uterine egg is a fully developed embryo, which breaks through the opercular opening to proceed with its development. This larva is not a miracidium, as characteristic for the DIGENEA, for it possesses the fundamental organization of the adult. It develops directly into an adult, either in the same host or in another individual of the same or different species. Parasitized UNIONIDAE when ingested by cold-blooded vertebrates such as fishes, frogs, or turtles, may liberate their worms in the stomach or intestine of the vertebrate. In the worms withstanding digestion, a potential second host becomes annexed to a primitively simple life cycle.

My findings concerning the life cycle of *Aspidogaster conchicola* give added support to the contention of Faust and Tang (1936) that *Aspidogaster* represents a subclass intermediate between the MONOGENEA and the DIGENEA to which the name ASPIDOGASTREA may be applied.

BIBLIOGRAPHY

- VON BAER, K. E. 1827 Beiträge zur Kenntniss der niedern Thiere, in Nova Acta Acad. Nat. Curios 13: 525-557.
FAUST, E. C. 1922 Notes on the excretory system in *Aspidogaster conchicola*. Tr. Am. Mic. Soc. 41: 113-117.

- 1932 The excretory system as a method of classification of digenetic trematodes. *Quart. Rev. Biol.* 7: 458-468.
- FAUST, E. C. AND CHUNG-CHANG TANG. 1936 Notes on new aspidogastrid species, with a consideration of the phylogeny of the group. *Parasitology* 28: 487-501.
- KELLY, H. M. 1899 Statistical study of the parasites of the Unionidae. *Bull. Illinois State Lab. Nat. Hist.* 5: 399-418.
- STAFFORD, J. 1896 Anatomical structure of *Aspidogaster conchicola*. *Zool. Jahrb., Abt. Anat.* 9: 476-542.
- STUNKARD, H. S. 1917 Studies on North American Polystomidae, Aspidogastridae, and Paramphistomidae. *Illinois Biol. Monogr.* 3: 1-114.
- WARD, H. B. 1918 Parasitic flatworms, in Ward and Whipple's *Fresh-water Biology*. Wiley, N. Y.

EXPLANATION OF PLATE

All figures are camera lucida drawings representing conditions in *Aspidogaster*. The lines indicating magnification for Figs. 4 to 10 have the value of 132 μ ; for Figs. 1 and 2, 100 μ ; and for Fig. 3, 200 μ .

ABBREVIATIONS

- X—groove in ventral sucker
 I—intestine
 P—pharynx
 Rt—rudimentary tail
 VS—ventral sucker
 OS—oral sucker
 PGS—primordial germ cells

FIG. 1. Newly hatched larva (Stage I); ventral view of stained whole mount in clarite.

FIG. 2. Lateral view of larva (advanced second stage), showing ventral sucker somewhat twisted to left so as to show opening.

FIG. 3. Ventral view of late larva (fourth stage), showing slit-like opening of the shallow pit in the ventral sucker.

FIG. 4. Sagittal section through fourth stage, to one side of median plane, showing location of groove (X) as ventral sucker migrates along the ventral body surface.

FIG. 5. Sagittal section through fourth stage, in median plane, showing location of groove (X) of ventral sucker.

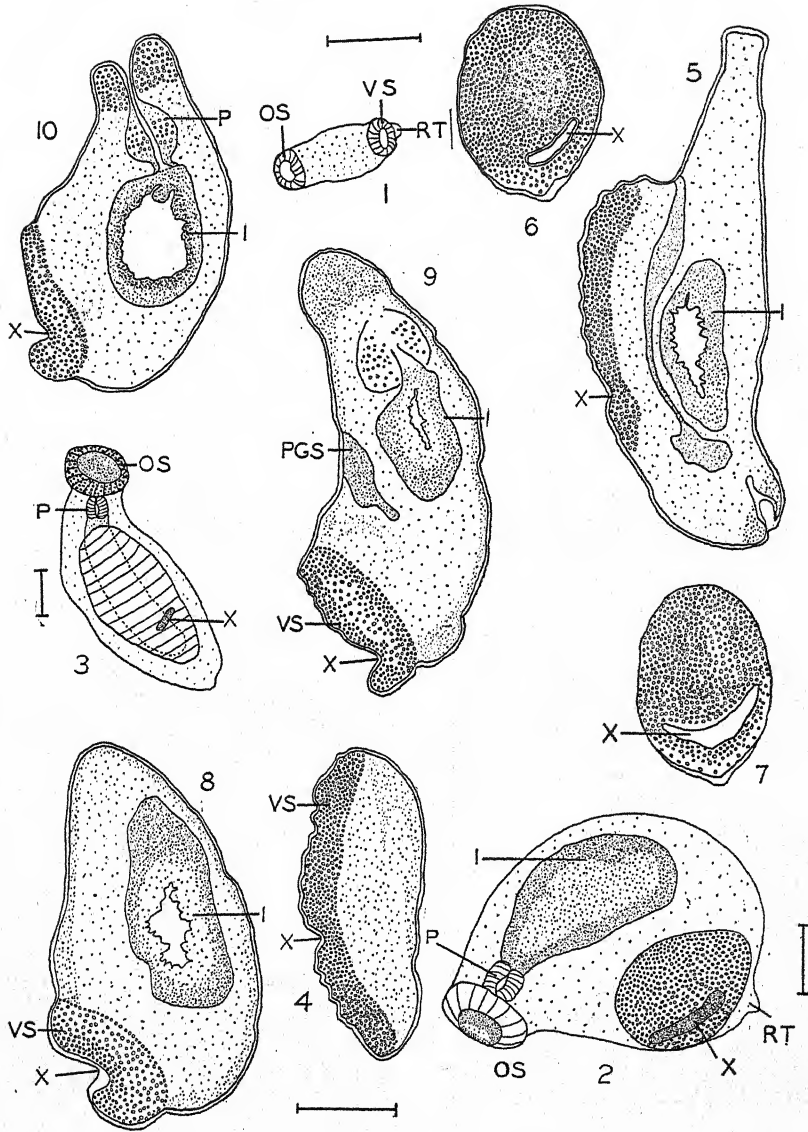
FIG. 6. Frontal section through ventral sucker of advanced second or early third stage, showing shape and position of slit-like opening of ventral sucker pocket.

FIG. 7. Frontal section, same as Figure 6 except that this section is taken ventral to Figure 6 on the same specimen.

FIG. 8. Sagittal section through third stage, showing ventral sucker and slit-like opening; to one side of median plane.

FIG. 9. Sagittal section through third stage, near median plane, showing ventral sucker, slit-like opening of ventral sucker, and posterior tip of ventral sucker which may be curled downward to increase depth of pocket of ventral sucker.

FIG. 10. Sagittal section through third stage, in median plane.



THE USE OF ROENTGEN RADIATION IN LOCATING AN
ORIGIN OF HOST RESISTANCE TO *TRICHINELLA SPIRALIS* INFECTIONS*

A. J. LEVIN AND T. C. EVANS

Departments of Zoology and Radiology, State University of Iowa,
Iowa City, Iowa

It has been demonstrated by Ducas (1), McCoy (2, 3), and Fischthal (4), and in experiments included in this investigation, that rats once infected with *Trichinella spiralis* may be highly resistant to subsequent infections. *Trichinella* infections consist of a primary intestinal phase and a secondary muscle invasion. The question has arisen as to whether the host resistance to re-infection is elicited by the penetration of the adult parasites into the intestinal mucosa, the migration of the larvae to the muscles, or both phases. This series of experiments was designed for the purpose of locating the origin of the resistance, and to demonstrate the use of roentgen radiation as a tool in resistance studies.

Tyzzer and Honeij (5), in 1916, Schwartz (6), in 1921, and Semrad (7), in 1937, demonstrated that radium or roentgen irradiation of *Trichinella* larvae, before feeding to a suitable host, would either destroy the larvae before they grew to maturity in the intestine of the host or else prevent the formation of young in the adult female worms that did develop. The extent of injury to the parasites was apparently proportional to the dosage of irradiation. The present authors (8, 9), employing quantitative methods, have determined the range of dosages of radiation which allows the trichina larvae to grow to maturity in the intestine without producing offspring. In this way, an intestinal infection without the consequent muscle invasion could be achieved. The aim of the present experiments is to determine whether an intestinal infection alone will produce resistance to a second infection of trichina.

MATERIALS AND METHOD

Trichinella larvae were removed from infected stock rats by pepsin digestion, and doses of approximately 4,000 larvae for each experimental rat were counted out by the dilution method. These larvae were transferred to small, celluloid dishes and irradiated with a given dose of roentgen radiation, either 3,250 r, 3,500 r, or 3,750 r (H.V.L. ca. 1.5 mm al., 427 r/m), and then fed to rats of the same age, with no previous infections. These were divided into three groups of six rats each, and

Received for publication, February 23, 1942.

* Aided by grants from the Committee on Radiations of the National Research Council and from the Rockefeller Fund for Research on the Physiology of the Cell.

one group of four rats. The group of four rats (Group I) was set up as a re-infection control. These rats received one infection of unirradiated larvae from the same source as the larvae used to re-infect the rats in Groups II, III, and IV.

Two weeks after the initial infection, all of the rats in Group II received re-infections of *Trichinella* which had not been irradiated, to determine whether a resistance to a second infection had developed. Half of the rats received a dose of approximately 2,500 larvae; the other half a dose of about 10,000. One week later (three weeks after the initial infection), the same procedure was carried out with the rats in Group III. On the fourth week after the initial infection, this procedure was again repeated with the rats in Group IV. Two controls (initially infected with unirradiated larvae from the same source as the larvae irradiated for experimental infections) were included in each group.

Two months after the last re-infection, the rats were killed, and dilution counts were made of the larvae removed from the rats' muscles by pepsin digestion.

RESULTS

The first problem to be met in designing these experiments was that of selecting the proper dosage of roentgen radiation. The desired dosage is that which will least affect the development of the *Trichinella* adults in the host intestine, while at the same time inhibit the formation of larvae destined to invade the muscle. Thus, an intestinal infection without the consequent muscle invasion can be accomplished.

After a few preliminary experiments had been performed, it was evident that the proper dosage would be somewhere in the neighborhood of 3,500 roentgens. Several experiments were then carried out using dosages of from 2,000 to 4,000 r, and the results are shown in Table 1. No second infections were given in these experiments. Based upon the evidence in Table 1, it was decided to use three dosages of radiation (3,250 r, 3,500 r, 3,750 r) for re-infection experiments in order to cover possible variations in the *Trichinella* larvae, in the host rats, and in the irradiation. However, it seems that all three dosages were equally effective in creating only intestinal infections.

The data in Table 2 are a summary of the results obtained by re-infecting rats with untreated larvae after they had first been given irradiated ones. Group I includes the re-infection controls, set up to test the viability of the larvae used for re-infection. The ratios given are an expression of the number of larvae recovered from the hosts' muscles divided by the number of larvae that caused the infection.

Group II includes the animals that were re-infected two weeks after the initial infection with irradiated larvae, the dose of radiation being indicated in the next column. The first two cases in this group, where

the initial infection larvae were not irradiated, serve as controls to confirm the findings of Ducas (1), McCoy (2, 3), and Fischthal (4); namely, that one infection causes a resistance to subsequent parasitic invasions. Our results are in accord with those of the above investigators.

In the two control cases marked with double asterisks (**), the low number of larvae recovered from the host muscle may be explained by the fact that the stock rat which served as the source of infective larvae was in the refrigerator for several days before the larvae were digested

TABLE 1.—Data from single infections each with approximately 4,000 irradiated *Trichinella* larvae. Rats of Group I (intestine) were sacrificed 5 days after infection. Rats of Group II (muscle) sacrificed after 2 months

Intestine (Group I)		Radiation dose (roentgens)	Muscle (Group II)	
No. adults recovered	Ratio*		No. larvae recovered	Ratio*
2,970	0.7	0	610,000	152.5
1,970	0.5	0	495,000	123.7
2,200	0.5	0	750,000	183.0
2,110	0.5	0	675,000	162.2
1,629	0.4	0	†	†
1,801	0.4	0	†	†
1,625	0.4	0	†	†
1,900	0.5	0	†	†
2,200	0.5	0	179,000	43.4†
1,550	0.4	2,000	50,500	12.6
1,475	0.3	2,000	3,100	0.7
2,010	0.5	2,000	2,250	0.6
2,280	0.6	2,000	†	†
2,475	0.6	3,000	564	0.1
700	0.1	3,000	‡	< 0.1
2,200	0.5	3,000	23	< 0.1
2,085	0.5	3,000	†	†
1,300	0.3	3,250	1	< 0.1‡
1,900	0.4	3,500	2	< 0.1
196	< 0.1	3,500	3	< 0.1
940	0.2	3,500	†	†
2,700	0.6	3,500	18	< 0.1‡
0	0.0	3,750	8	< 0.1
340	< 0.1	3,750	†	†
1,500	0.4	3,750	9	< 0.1‡
0	0.0	4,000	5	< 0.1
135	< 0.1	4,000	†	†
10	< 0.1	4,000	7	< 0.1

* The ratio expresses the number of worms recovered divided by the number of larvae fed to the rats.

† These rats were destroyed through accident.

‡ The larvae used for infection in these cases were from the same source as those used for the re-infection experiments similarly marked (†) in Table 2.

< 0.1 = less than 0.1.

out. Since these larvae were the only ones available at the time, they had to be used. It seems to have made no difference as far as setting up resistance in the experimental animals. Some of the larvae may have been devitalized, as the number of worms recovered in each of the two cases is small. However, the larvae used for the second infection were not refrigerated and produced a heavy infection in a rat not previously treated (Table 2, Group I, initial infection 9,600). Thus, the small number of worms recovered in these two control cases indicates that the

initial, and not the second infection is reflected in the number of larvae in the muscles. This is in accord with the data compiled by Ducas (1), McCoy (2, 3), and Fischthal (4). In computing ratios of these and other controls in Groups III and IV, Table 2 (0 irradiation), the number of larvae recovered is divided by the number of larvae used for the initial infection (or the larvae causing the infection).

However, in computing the ratios for the experimentals, the number of larvae recovered from the muscles was divided by the number of larvae re-infected because the data from Table 1 indicate that the initial dose

TABLE 2.—Data from re-infection experiments

Group	Initial infection (larvae)	Radiation dose (roentgens)	Re-infection dose (larvae)	No. muscle larvae recovered	Ratio*
I (re-infection control)	9,600	411,000	42.8
	2,500	165,000	66.0
	2,920	(Note a)	..
	4,120†	179,000	43.4
II (re-infected 2 weeks after initial infection)	3,900	0	9,920	16,000	**
	3,900	0	2,480	44,000	**
	3,900	3,250	9,920	30	< 0.1
	3,900	3,250	2,480	1,017	0.4
	3,900	3,500	9,920	3,682	0.4
	3,900	3,500	2,480	846	0.3
	3,900	3,750	9,920	75	< 0.1
	3,900	3,750	2,480	103	< 0.1
III (re-infected after 3 weeks)	3,900	0	10,000	147,000	37.7
	3,900	0	2,500	149,000	38.1
	3,900	3,250	10,000	115	< 0.1
	3,900	3,250	2,500	27	< 0.1
	3,900	3,500	10,000	23	< 0.1
	3,900	3,500	2,500	230	< 0.1
	3,900	3,750	10,000	2,800	0.3
	3,900	3,750	2,500	58	< 0.1
IV (re-infected after 4 weeks)	4,120†	0	10,220	157,000	38.1
	4,120†	0	2,920	276,000	67.0
	4,120†	3,250	10,220	45,000	4.4
	4,120†	3,250	2,920	5,200	1.8
	4,120†	3,500	10,220	847	< 0.1
	4,120†	3,500	2,920	36,500	12.5
	4,120†	3,750	10,220	4,000	0.4
	4,120†	3,750	2,920	30	< 0.1

Note a: This rat died of trichinosis three weeks after infection.

* The ratio expresses the number of larvae recovered from the muscle divided by the number of larvae causing the infection. Refer to Results.

** Explanation of these two cases will be found in Results.

† The larvae used for the initial infection in these cases are from the same source as the larvae similarly marked (‡) in Table 1.

< 0.1 = less than 0.1.

of irradiated larvae are not responsible for the trichinae recovered from the muscles, provided the irradiation dose is large enough to destroy all the germ cells in each female. This apparently happens with irradiation doses above 3,250 r (Table 1).

The re-infection doses (Table 2) were divided into light (2,500 larvae) and heavy doses (10,000 larvae), to determine if a heavy infection would break down this intestinal resistance. No appreciable difference in resistance against light or heavy doses was noted.

DISCUSSION

In 1916, Tyzzer and Honeij (5) treated *Trichinella* larvae encysted

in muscle with radium and found that these irradiated larvae produced no infection in mice. Schwartz (6), in 1921, irradiated trichina larvae encysted in pork, and then fed the pork to rats. He observed that the irradiation either destroyed the larvae before they grew to maturity in the intestine of the host, or prevented the formation of young in the adult female worms. This was due, he stated, to the fact that the X-rays exerted a selective action on the sex cells of trichina without necessarily affecting the other vital organs. The sex cells of the adult appeared to be atrophied, no spermatozoa were found in the receptaculum seminis of the females, and no evidence of successful copulation could be found.

Semrad (7), in 1937, repeated these experiments, using more exact doses of roentgen radiation. He found that as the dose of radiation was increased, from 400 r to 2,000 r, the average number of larvae in 12 square millimeters of diaphragm decreased markedly. The present authors (8, 9) repeated Semrad's experiments, using a greater range of radiation, and recovery of larvae in all of the muscle tissue. The same trend was found to be true. In addition, it was found that by selecting a certain range of radiation (3,250 r to 3,750 r) it was possible to obtain survival of the larvae in the intestine, and at the same time prevent any material number of offspring from being produced. To determine if an intestinal infection alone could grant host resistance to a second infection, rats (infected with an initial dose of irradiated larvae) were re-infected with untreated larvae. From the data summarized in Table 2, it is evident that a definite resistance to a second infection of *Trichinella* is produced in rats by the initial feeding of irradiated larvae.

It appears that the acquired immunity to certain helminths is directed largely against the intestinal phase of the parasite, and the mechanisms involved may be an increased secretion of mucus and increased peristalsis (McCoy, 3; Ackert, 12). Several workers have failed to demonstrate a passive immunizing action of infected host serum and most of these, McCoy (3), Chandler (10, 14), and Spindler (11), consider the immunity as being localized in the intestine. Other investigators such as Sheldon (15), Sarles and Taliaferro (16), Culbertson and Kaplan (17), Culbertson (18), and Bachman (19) believe that the initial infection produces a generalized immune response characterized by the production of antibodies. While the scope of this paper does not permit a discussion of the different aspects of the subject of immunity, the data presented herein indicate that the passage of larvae through the vascular system and the invasion of the muscles are not necessary for the development of a resistance to a second infection with *Trichinella spiralis*. Further information on the possibility of the production of a generalized immune response might be gained by resistance studies on parabiotic rats in which one of each pair was first infected with properly irradiated larvae.

As a practical application of the procedure employed herein, the authors wish to suggest the possibility of making young pigs resistant to trichinosis (by feeding them with properly irradiated *Trichinella* larvae) as a means of eradicating human infection. Account should be taken of the differences in reaction to the parasite on the part of the pig and rat hosts, so that further careful investigation should be carried on before applying any of the irradiation and larvae doses (as given here) on a large scale. Although the technical difficulties involved present obstacles, the authors do not feel that they are unsurmountable.

The inhibition of the reproductive capacity of parasites by roentgen radiation seems to be a very effective method of studying host resistance mechanisms, and its application to other parasitic forms is worthy of further investigation. It may be noted that Waxler and Herrick (20) have achieved success in immunizing chickens against coccidiosis with this method. Waxler and Herrick speak of this irradiation effect as attenuation, but further work is undoubtedly needed to determine the exact mechanisms involved. Their procedure has certain similarities to the experiments herein reported, and the results might possibly be due to the inhibition of reproductive stages of the parasite following the irradiation.

The suggestions given above are made in the hope that others will take up this work, utilizing roentgen radiation as a tool in the study of host resistance to parasitic infections.

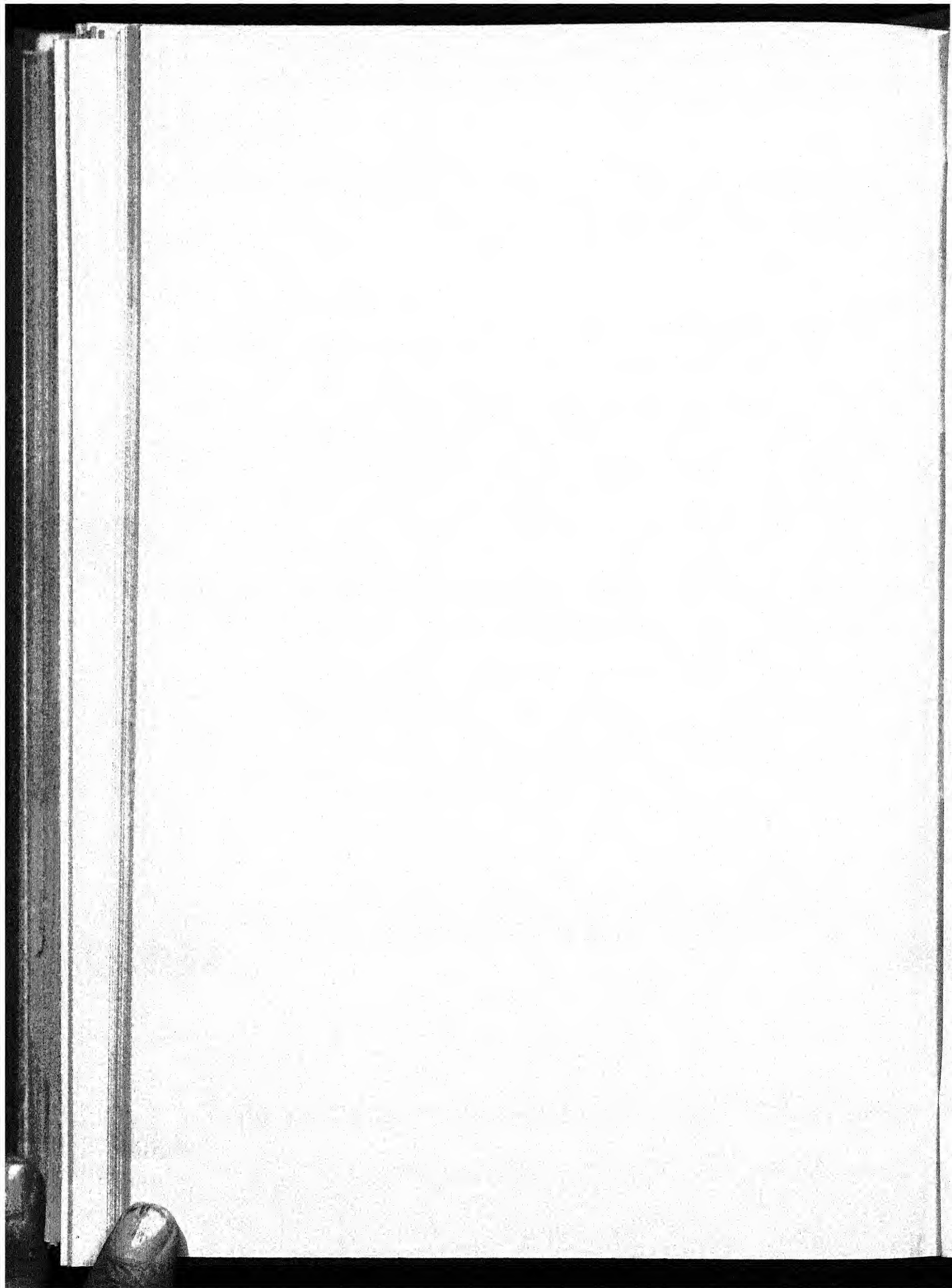
SUMMARY

1. It is possible to treat *Trichinella spiralis* larvae with roentgen radiation before infecting rats, so that the larvae grow to maturity in the intestine but produce no offspring. In this way, an intestinal infection without the consequent muscle invasion can be achieved.
2. This intestinal infection alone will produce strong host resistance to a second infection of *Trichinella*.
3. Since an intestinal infection alone will produce resistance to a second infection, it is concluded that the origin of a mechanism of host resistance to *Trichinella* is located in the intestine.
4. Theoretical and practical considerations of intestinal resistance are discussed, and suggestions are made for further investigations.
5. The use of roentgen radiation as a tool for the study of host resistance to parasitic infections is urged.

BIBLIOGRAPHY

1. DUCAS, R. 1921 L'immunité dans la trichinose. These, Paris, Jouve et Cie. P. 47.
2. MCCOY, O. R. 1931 Immunity of rats to reinfection with *Trichinella spiralis*. Am. J. Hyg. 14: 484-494.

3. ————1940 Rapid loss of *Trichinella* larvae fed to immune rats and its bearing on the mechanism of immunity. *Am. J. Hyg.* 32 D: 105-116.
4. FISCHTHAL, J. H. 1938 Intestinal infestation with *Trichinella spiralis*. Thesis, State University of Iowa. (Unpublished.)
5. TYZZER, E. E. AND HONEIJ, J. A. 1916 The effects of radiation on the development of *Trichinella spiralis*. *J. Parasitol.* 3: 43-56.
6. SCHWARTZ, B. 1921 Effects of x-rays on trichinae. *J. Agr. Res.* 20: 845-854.
7. SEMRAD, J. E. 1937 Effects of roentgen radiation on trichinosis in the albino rat. *Am. J. Roentgenol. and Rad. Ther.* 38: 470-477.
8. LEVIN, A. J. AND EVANS, T. C. 1940 Effect of roentgen radiation on embryonic development of *Trichinella spiralis*. *J. Parasitol.* 26 Suppl: 31.
9. EVANS, T. C., LEVIN, A. J. AND SULKIN, N. M. 1941 Inhibition of embryo formation in certain nematodes by roentgen radiation. *Proc. Soc. Exper. Biol. and Med.* 48: 624-628.
10. CHANDLER, A. C. 1932 Susceptibility and resistance to helminthic infections. *J. Parasitol.* 18: 135-152.
11. SPINDLER, L. A. 1934 Resistance of rats to superinfection with *Nippostrongylus muris*, following administration of adult worms by duodenal tube. *J. Parasitol.* 20: 326.
12. ACKERT, J. E. 1942 Natural resistance to helminthic infections. *J. Parasitol.* 28: 1-24.
13. ANDERSON, C. V. AND LEONARD, A. B. 1940 Immunity produced in rats by the intestinal phase of *Trichinella* infection. *J. Parasitol.* 26 Suppl: 42.
14. CHANDLER, ASA C. 1935 Studies on the nature of immunity to intestinal helminths. I. The local nature of the immunity of white rats to *Nippostrongylus* infection. *Am. J. Hyg.* 22: 157-168.
15. SHELDON, A. J. 1936 Studies on active acquired resistance, natural and artificial, in the rat to infections with *Strongyloides ratti*. *J. Parasitol.* 22: 533.
16. SARLES, M. P. AND TALIAFERRO, W. H. 1936 The local points of defense and the passive transfer of acquired immunity in experimental trichiniasis. *J. Infect. Dis.* 59: 207-220.
17. CULBERTSON, J. T. AND KAPLAN, S. S. 1937 Passive immunity in experimental trichiniasis. *J. Parasitol.* 23: 560.
18. CULBERTSON, J. T. 1942 Passive transfer of immunity to *Trichinella spiralis* in the rat. *J. Parasitol.* 28: 203-206.
19. BACHMAN, G. W. 1929 A precipitin test in experimental trichiniasis. *J. Prev. Med.* 3: 465-469.
20. WAXLER, S. H. AND HERRICK, C. W. 1941 Immunization against coccidiosis by the use of x-ray attenuated oöcysts. *J. Parasitol.* 27 Suppl: 17.



REMARKS ON THE TAXONOMY OF SOME AMERICAN
CHIGGERS (TROMBICULINAE), INCLUDING
THE DESCRIPTIONS OF NEW
GENERA AND SPECIES

H. E. EWING

Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture

Chiggers are parasitic larvae of mites of the subfamily TROMBICULINAE and infest vertebrates exclusively. They are distributed throughout most of the temperate and tropical regions of the world. Up to the present there have been reported from the Western Hemisphere 10 valid genera and 76 valid species. To this number there are here added four genera, two of which are new, and seven species, all being new. There probably remain to be described from the New World at least twice as many genera and species as have been already reported.

THE PROPER SCIENTIFIC NAME FOR THE COMMON NORTH
AMERICAN CHIGGER

For many years the common North American chigger was known by the scientific name *Leptus irritans*, given to it by C. V. Riley (1873). It was pointed out by Oudemans (1937), however, that the name *Leptus irritans* had been used previously by Lucas (1847) for a "patatta" mite, or chigger, occurring in Brazil. Following this disclosure the writer (Ewing, 1938a, p. 26) reviewed the synonymy of the species and came to the conclusion that *alfreddugèsi* (Oudemans) was the oldest valid name for *irritans* Riley.

When the writer's key to the genera of chiggers was published (Ewing, 1938b), *alfreddugèsi*, which had been described under the generic name *Microtrombidium* and which for some years had been placed in *Trombicula*, was made the type species of a new genus, *Eutrombicula*. Thus the scientific name of our most common and best known chigger became *Eutrombicula alfreddugèsi* (Oudemans). But Oudemans has recently proposed *Leptus rileyi* (Oudemans, 1939) as a new name for *Leptus irritans* Riley. This not only was unnecessary, since there already existed several synonyms of Riley's species, but became confusing since some workers adopted his new name as the proper one to apply to Riley's *irritans* at the present time. If Oudemans were correct in not recognizing his *alfreddugèsi* as being the same as Riley's *irritans*, then the proper name to apply to the latter species would probably be my *cinnabaris* (Ewing, 1921, p. 387) which undoubtedly is the same as Riley's *irritans* but may not have priority over Hirst's *similis* (Hirst, 1921). Although

Received for publication, March 4, 1942.

the printed date for *cinnabaris* is December 1920, the actual date of publication was some time in 1921. The writer sees no good reason for not using the specific name *alfreddugèsi* for Riley's *irritans*.

THE STATUS OF *Trombicula hirsti* SAMBON

Sambon (1927) described under the above name a man-infesting chigger from North Queensland, Australia, known locally as "the scrub itch-mite." According to the description and excellent drawings (made by Terzi) of this species it agrees with our North American *alfreddugèsi* in all respects except one, i.e., the inner prong of the palpal claw in *hirsti* is stouter than the outer, while in *alfreddugèsi* the reverse is true.

Womersley (1939) regards Sambon's species as only a synonym of *Trombicula minor* Berlese, the genotype of *Trombicula*. He states: "I have now received from Dr. W. G. Heaslip an adult female (of *Trombicula hirsti* v. *buloloensis* Gunther) found at Innisfail in Queensland, December, 1939, which also corresponds to Berlese's species (*Trombicula minor*). As the only larval *Trombicula* known from Queensland is *T. hirsti* Sambon, the common itch-mite of that State, the above correction is further confirmed." If this synonymy is correct, *Eutrombicula* Ewing becomes a synonym of *Trombicula* Berlese, and the Japanese chigger, *Trombicula akamushi* (Brumpt), and the autumnal chigger of Europe, *Trombicula autumnalis* (Shaw), belong to *Leptotrombidium* Nagayo and other authors, since both species have a trifurcate palpal claw.

A QUILL-INFESTING CHIGGER

Torres and Braga (1938) have described a Brazilian chigger of unusual habits. This chigger, which has the common name of "micuim", was placed in a new genus, *Apolonia*, and described as new, being given the name *tigipioensis*. According to the authors, the chigger enters the quills of very new feathers of chickens and produces nodules and lesions. They give a photograph of a fowl showing the presence of these nodules on the upper part of each leg.

Torres was in Washington recently and left with this writer a sample of one of these nodules and many infested quills for study. The nodule was about one-half the size of a pea and each infested quill had broken off near its base. In the short, broken-off, basal part of each was found a single chigger. The chigger usually completely filled the lumen of the quill at the latter's base, and always was oriented so as to face the basal end of the quill.

Several of the specimens left by Torres have been cleared and mounted in a modified Berlese mixture. They exhibit the essential taxonomic characters of *Apolonia tigipioensis*, as given by Torres and Braga. If this quill-infesting chigger spreads to other countries, it may become a poultry pest of major importance.

THE NATURAL AFFINITIES OF *Apolonia*

In a second paper, Torres and Braga (1939) have placed *Apolonia* next to *Leeuwenhoekia* Oudemans in a key adapted from Ewing (1929, p. 22). In this they are correct, according to the opinion of the writer. Even in the revised key to genera (Ewing, 1938b), this new genus should be placed next to *Leeuwenhoekia*. It differs from the latter particularly in the loss of the posterolateral setae of the dorsal plate.

Paraschöngastia

Womersley (1939) proposed a new genus, *Paraschöngastia*, for four New Guinea species of *Neoschöngastia*, but failed to name a genotype. According to his diagnosis of *Paraschöngastia*, the contained species differ from the remaining ones of *Neoschöngastia* particularly "in that there is a distinct raised crest in front of the pseudostigmata." Some of our American species of *Neoschöngastia* are very close to those included in *Paraschöngastia*.

NEW GENERA AND SPECIES

There are here described two new genera and seven new species of chiggers. One of the new species, *Schöngastia lynni*, probably will be made the type species of a new genus in the future, when more material is obtained. All the specimens of this species at hand are injured, in that the pseudostigmatic organs have been detached.

Schöngastia lynni n. sp.
(Figs. 1, 2)

Chelicera obliquely flattened at distal end so as to form a posteriorly directed process which is toothed on its margin; ventral tooth large, flattened, directed backward. Palpus not肘ed but rounded laterally; first seta with 2 to 4 short, lateral branches; second seta either simple or with a single, short, lateral branch; palpal claw slightly cleft distally, forming 2 small, sharply pointed, lateral prongs closely appressed to the much larger middle element. Eyes of a side situated on a poorly sclerotized ocular plate; anterior eye slightly smaller than posterior and either granular or pigmented underneath cornea. Dorsal plate about twice as broad as long and shaped as shown in Fig. 2; setae all slightly curved and indistinctly barbed, the median and posterolateral subequal and longer than the anterolateral; pseudostigmatic organs detached from each specimen. Dorsal abdominal setae about 30, slightly curved, indistinctly barbed. The shoulder pair present, followed by a transverse row of 6, then a transverse row of 4, the remainder not arranged in distinct rows. Legs of moderate length, each composed of 7 segments and provided with the usual tarsal armature.

Length of partly engorged larva, 0.63 mm; width, 0.41 mm.

Type host.—"On frog."

Type locality.—Blue Mountains, Jamaica, West Indies.

Type slide (holotype).—U. S. N. M. No. 1412.

Description based on four specimens taken by Dr. Lynn. This species has the chelicerae of *Hannemania* and the dorsal plate of several other genera. It would have been assigned a new genus if the pseudostigmatic

organs had not been detached from every specimen. The writer has been very reluctant regarding the establishment of a new genus upon a species all known representatives of which are without a certain structure of generic value. In the case of *S. lynni*, the loss of the pseudostigmatic organs deprives us of one of the most important generic characters and makes it impossible to place a new genus, established on it, properly in a key.

Trombicula rohweri n. sp.

(Fig. 3)

Chelicera short, stout; dorsal and ventral tooth each very sharp and barblike. Palpus subangulate laterally; first and second palpal setae plumose; inner prong of palpal claw usually more slender, less curved, and more closely appressed to middle one than outer prong; middle prong longer and stouter than the other 2. Eyes 2 on each side, subequal, situated on a thick, strongly sclerotized, ocular plate; anterior eye with granulations and pigment. Dorsal plate (Fig. 3) minutely pitted except along anterior margin and about the base of median seta; behind each pseudostigma is a transverse ridge which may possibly be accompanied by a concealed transverse slit. Dorsal abdominal setae 36 to 40, curved, weakly plumose, arranged in irregular transverse rows. Legs of medium length, seven-segmented; coxae each with enlarged and heavily sclerotized distal margin.

Length of partly engorged larva, 0.47 mm; width, 0.30 mm.

Type host.—Eastern house wren, *Troglodytes aedon aedon*.

Type locality.—Hindsboro, Mississippi.

Type slide (cotypes).—U. S. N. M. No. 1413.

Description based on four cotypes taken February 14, 1940, by G. G. Rohwer.

This species is closely related to *T. oregonensis* Ewing, from which it differs in having a much narrower dorsal plate and a transverse ridge behind each pseudostigma. It differs from *T. californica* n. sp., in having the first palpal seta plumose instead of simple.

Trombicula californica n. sp.

(Figs. 4, 5)

Chelicera short, stout; dorsal and ventral tooth each a minute barb. Palpus not angulate laterally; first and second setae rather short, simple; outer prong of palpal claw usually stouter than inner and not so closely appressed to the middle prong, which is the largest and most strongly curved of the three. Eyes 2 on each side; situated on ocular plate; anterior eye slightly smaller than posterior and somewhat granular under cornea. Dorsal plate slightly broader than long and shaped as shown in Fig. 4; almost the entire surface minutely pitted. Dorsal abdominal setae about 40, slightly curved, weakly plumose; 10 in the first and 10 in the second transverse rows. Legs rather short, each composed of 7 segments and provided with the usual tarsal armature.

Length of partly engorged larva, 0.40 mm; width, 0.28 mm.

Type host.—*Thomomys neglectus*.

Type locality.—Acton, California.

Type slide (cotypes).—U. S. N. M. No. 1414.

This species belongs to the *whartoni* group and is most nearly related to *Trombicula oregonensis* Ewing, from which it differs in having the first

palpal seta simple instead of branched and the dorsal plate slightly broader than long instead of about twice as broad as long. California material at hand as follows: Seven specimens from type host and type locality, April 12, 1933, taken by A. G. Barr; nine specimens on *Thomomys* species, Fresno, July 6, 1932, taken by B. P. Bole, Jr.; four specimens on *Microtus californicus sanctidiegi* and two specimens on "wood rat," Los Angeles County, January 9, 1933, taken by M. F. Canova. British Columbia material as follows: One specimen on *Peromyscus* species, Vancouver, January 14, 1937, from G. J. Spencer.

Comatacarus n. g.

Chelicera with but a single dorsal and a single ventral tooth; chelicera not obliquely flattened distally. Palpus with claw two- or three-pronged, either the first or second seta, or both, plumose or barbed. Eyes present. Dorsal plate entire, moderately sclerotized; crista absent; anterior median process present; pseudostigmatic organs flagelliform, simple; anterolateral setae large, plumose; posterolateral setae not clavate but similar in form to anterolateral; median seta absent; paramedian setae present, similar to anterolateral setae; lateral setae absent. Dorsal abdominal setae similar, sessile, curved, weakly plumose and numerous. Tarsi each armed distally with 3 claws, the outer 2 being equal, strongly curved and stouter than the more slender, longer, and less curved median claw.

Type species.—*Comatacarus americanus* n. sp.

Comatacarus is erected to include a few species which have the characters of *Leeuwenhoekia* Oudemans (1911), it being desired to divide Oudemans' genus into three genera. It differs from *Leeuwenhoekia*, sensu strictu, in having the posterolateral setae of the dorsal plate of the usual form and similar to the anterolateral, instead of being clavate, and in having a large number of sessile setae on the dorsum of the abdomen, instead of a small number situated on the tubercles. Besides the type species, one other from the New World is included, *C. occidentalis* n. sp. Probably one or more of the Old World species will later be referred to this genus.

Comatacarus occidentalis n. sp.

(Fig. 6)

Chelicera narrowed distally and formed into a more or less dartlike head. Palpus not angulate laterally; first seta weakly plumose; second seta simple; palpal claw two-pronged, outer prong much more slender, slightly shorter, and less curved than inner. Eyes equal, almost contiguous, each with a well-formed cornea. Dorsal plate smooth, about twice as broad as long; anterior median process angulate apically; submedian setae weakly plumose, almost straight, and slightly shorter than similar anterolateral setae; posterolateral setae similar to anterolateral but much longer; pseudostigma situated on a short, transverse ridge; pseudostigmatic organ long, simple, flagelliform. Dorsal abdominal setae curved, weakly plumose, and rather closely studding the dorsum, over 80 in number, and arranged in somewhat irregular rows. Legs six-segmented; coxa II more slender than other 2.

Length of unengorged larva, 0.32 mm; width, 0.19 mm.

Type host.—"On common ground squirrel."

Type locality.—Lake Pillsbury, California.

Type slide (holotype).—U. S. N. M. No. 1415.

Description based on holotype, the only specimen at hand, collected September 10, 1939, by Deonier, Bishopp and Lindquist. This species is easily distinguished from its congener, the description of which immediately follows, by having the second palpal seta simple instead of weakly plumose.

Comatacarus americanus n. sp.

(Fig. 7)

Chelicera stout, strongly upcurved at and near apex; dorsal and ventral tooth small, each situated nearer than usual to apex of chelicera. Palpus rather short, rounded laterally; first seta curved, plumose; second seta straight, longer than first, plumose; palpal claw three-pronged, middle prong very much stouter and somewhat longer than the very sharp, almost straight, subequal lateral prongs. Anterior and posterior eyes contiguous, situated on a short, broad, oval ocular plate. Dorsal plate smooth; other characters as shown in Fig. 7. Dorsal abdominal setae more than 80, slightly curved, plumose, 12 setae in first transverse row. Legs six-segmented; coxa II longer but not so broad as coxa III.

Length of partly engorged larva, 0.41 mm; width, 0.27 mm.

Type host.—"Western mole."

Type locality.—Portland, Oregon.

Type slide.—U. S. N. M. No. 1416.

Description based on two lots: Six specimens from type host and type locality May 20, 1936, taken by C. M. Gjullin and one specimen on "cotton mouse," Dale County, Alabama, April 5, 1937, from R. E. Dyer.

Acomatacarus n. g.

Most nearly related to *Comatacarus* but differing from it as follows: Chelicera with a row of teeth on upper margin instead of a single tooth and both the first and second palpal setae simple instead of one or both being plumose or barbed.

Type species.—*Acomatacarus arizonensis* n. sp.

Of the New World chiggers only the type species is included. Of the Old World species several should be included, three of which are *Leeuwenhoekia polydiscum* Oudemans, *L. jaegerskioeldi* Oudemans, and *L. australiensis* Hirst.

Acomatacarus arizonensis n. sp.

(Figs. 8, 9)

Chelicera rather slender, bearing above from 3 to 5 unequal teeth. Palpus rounded laterally; first and second setae subequal, curved; palpal claw strongly curved and unequally divided, outer prong much smaller and more sharply pointed than inner. Eyes not situated on ocular plate, anterior slightly larger than, and almost contiguous with, the posterior. Dorsal plate (Fig. 9) very broad, smooth. Pseudostigma peculiar in that it is somewhat overlapped in front by a liplike ridge. Dorsal abdominal setae between 40 and 50, rather short, curved, very weakly plumose, arranged in indefinite transverse rows. Legs of medium length, six-segmented, although segments II to V (inclusive) each possesses a pigmented band indicating a false division.

Length of unengorged larva, 0.22 mm; width, 0.12 mm.

Type host.—A lizard, *Callisaurus draconoides ventralis*.

Type locality.—Near Wickenburg, Arizona.

Type slide.—U. S. N. M. No. 1417.

Description based on six specimens taken from type host at type locality, May 11, 1937, by Roy Komarek.

Walchia americana n. sp.
(Figs. 10, 11)

Chelicera stout; upper tooth larger than usual, not recurved or barblike; lower tooth directed backward, acutely angulate at apex. Palpus short, subangulate laterally; first seta short, slightly curved, simple; second seta subequal and similar to first; 3 prongs of palpal claw similar, but middle prong usually larger than others. Eyes absent. Dorsal plate smooth; a short, curved ridge in front of each pseudostigma; pseudostigmatic organs missing from all specimens, probably being detached by the treatment in caustic before mounting; for other characters of dorsal plate see Fig. 11. Dorsal abdominal setae about 26, very slightly curved, each with a few minute lateral branches or barbs. Number of setae in first 4 transverse rows as follows: 6, 6, 4, 4. Legs very short; those of first pair seven-segmented (femur divided), those of other pairs six-segmented; coxa II longer and more slender than other 2.

Length of unengorged larva, 0.24 mm; width, 0.13 mm.

Type host.—"On cotton mouse."

Type locality.—Tallahassee, Florida.

Type slide (cotypes).—U. S. N. M. No. 1418.

Description based on nine specimens taken on type host from type locality, November 8, 1936, by B. V. Travis. This species is placed in *Walchia* with some hesitation. It is closely related to *Gahrlepiea turmalis* Gater of the Federated Malay States, from which, however, it differs in having the dorsal plate of a different shape. Like *G. turmalis*, *W. americana* has the first and second palpal setae short, curved, and simple, and femur I divided, yet unlike *G. turmalis* it has a short, curved ridge near each pseudostigma as is the case in *Walchia lewthwaitei* Gater.

BIBLIOGRAPHY

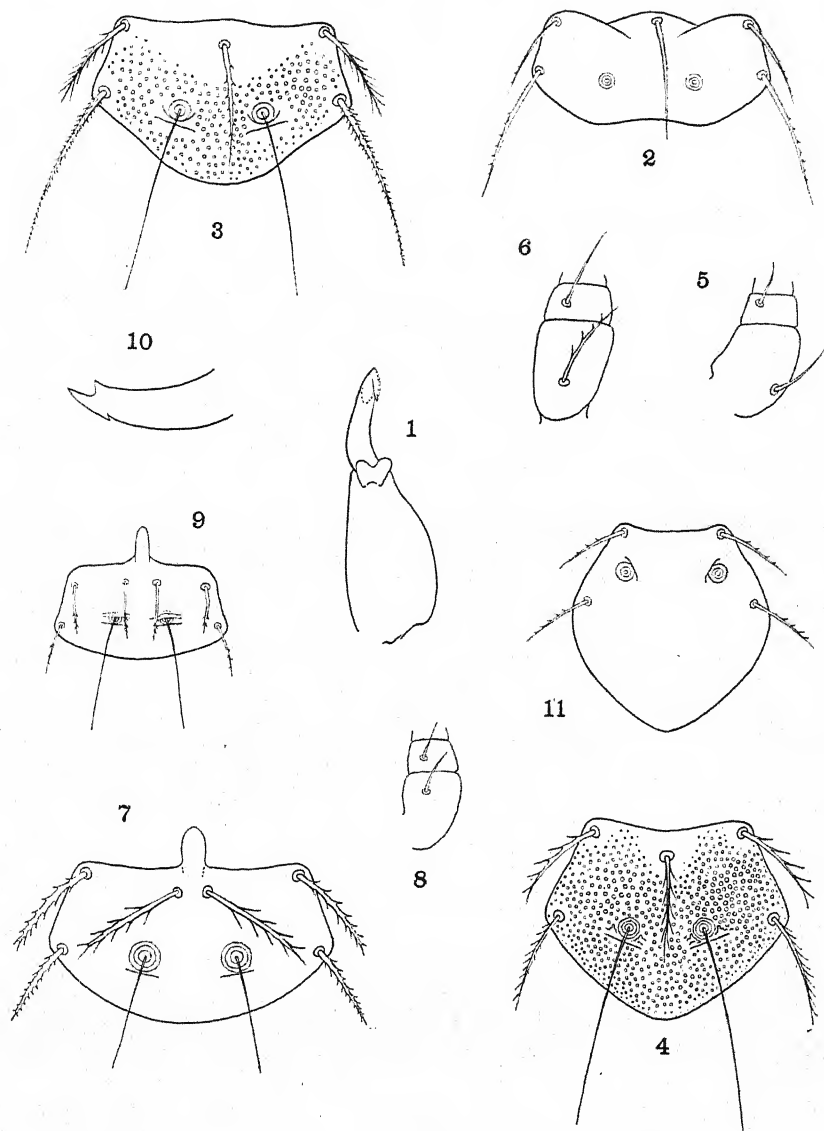
- EWING, H. E. 1921 The genus *Trombicula* Berlese, in America and the Orient. *Ann. Entom. Soc. Am.* 13: 381-390.
 ——— 1929 A Manual of External Parasites. Charles C. Thomas, Springfield, Ill. 225 pp.
 ——— 1938a The scientific name of the common North American chigger preoccupied. *Proc. Helm. Soc. Washington* 5: 26-27.
 ——— 1938b A key to the genera of chiggers (mite larvae of the subfamily Trombiculinae) with descriptions of new genera and species. *J. Wash. Acad. Sc.* 28: 288-295.
 HIRST, S. 1921 On three new parasitic mites (*Leptus*, *Schöngastia*, and *Demodex*). *Ann. and Mag. Nat. Hist.* (9)7: 37-39.
 LUCAS, H. 1847 Sur des Acariens Trouvés au Para. *Bul. Soc. Entom. France* (2)5: xxxvi-xxxvii.
 OUDEMANS, A. C. 1937 Kritisch Historisch Overzicht der Acarologie. Pt. 3, Vol. D, p. 1389.
 ——— 1939 Neue Funde auf dem Gebiete der Systematik und der Nomenklatur der Acari. VI. *Zool. Anz.* 127: 75-80.
 RILEY, C. V. 1873 Harvest mites. *Am. Naturalist* 7: 16-19.
 SAMBON, L. W. 1927 The "scrub itch-mite" of North Queensland: a new species of *Trombicula*. *Ann. and Mag. Nat. Hist.* (9)20: 157-161.
 TORRES, S. AND BRAGA, W. 1938 Nova parasitose em pintos. *Trab. Sec. Veter. Inst. Pesq. Agron. Pernambuco*, pp. 171-172.

- AND ——— 1939 *Apolonia tigipiöensis*, g. et sp. n. (Trombiculinae) parasito de *Gallus gallus* dom. Bol. Soc. Bras. Med. Vet. 9: 1-7.
- WOMERSLEY, H. 1939 Further notes on the Australian Trombidiidae with descriptions of new species. Tr. and Proc. Roy. Soc. South Australia 63(2): 149-166.

EXPLANATION OF PLATE

All drawings of the dorsal plate are of the same magnification, the others are differently and more highly magnified.

- FIG. 1. *Schöngastia lynni* n. sp.; dorsal view of chelicera.
- FIG. 2. *Schöngastia lynni* n. sp.; dorsal view of dorsal plate. (Pseudostigmatic organs probably detached by process of clearing specimens.)
- FIG. 3. *Trombicula rohweri* n. sp.; dorsal view of dorsal plate.
- FIG. 4. *Trombicula californica* n. sp.; dorsal view of dorsal plate.
- FIG. 5. *Trombicula californica* n. sp.; dorsal view of basal part of palpus.
- FIG. 6. *Comatacarus occidentalis* n. sp.; dorsal view of basal part of palpus.
- FIG. 7. *Comatacarus americanus* n. sp.; dorsal view of dorsal plate.
- FIG. 8. *Acomatacarus arizonensis* n. sp.; dorsal view of basal part of palpus.
- FIG. 9. *Acomatacarus arizonensis* n. sp.; dorsal view of dorsal plate.
- FIG. 10. *Walchia americana* n. sp.; side view of piercing arm of chelicera.
- FIG. 11. *Walchia americana* n. sp.; dorsal view of dorsal plate.



RESEARCH NOTES

THE MOURNING DOVE, A NEW HOST OF THE ANOPLOCEPHALID TAPEWORM, *APORINA DELAFONDI* (RAILLIET)

Thirty-one mourning doves, *Zenaidura macroura carolinensis* (L.), from Wake County, North Carolina, were examined for parasites during the spring of 1940. Five of them were infected with mature specimens of the tapeworm, *Aporina delafondi* (Railliet). One host harbored three specimens and the other four were parasitized with one each. This report appears to be the first record of this species infecting the mourning dove. It has been recorded by Cram (1924, J. Parasitol. 11: 115) and Hussey (1941, Am. Midland Naturalist 25: 413-417) as infecting the pigeon in the United States.—REINARD HARKEMA, Zoology Department, North Carolina State College, Raleigh, N. C.

AN IMPROVED TECHNIQUE FOR THE STUDY OF THE ACANTHOR STAGE IN CERTAIN ACANTHOCEPHALAN LIFE HISTORIES

Manter (1928, Tr. Am. Micr. Soc. 47: 342-347) described a simple method by which he was able to induce artificial hatching of acanthocephalan eggs. By allowing a culture of eggs, from the body cavity of a mature female worm, to dry completely in a Syracuse watch glass and then re-wetting with water he found that a number of eggs hatched immediately, releasing the first larval stage, the acanthor. Manter used the method successfully with the eggs of *Macracanthorhynchus hirudinaceus* and *Mediorhynchus* sp., but he was not able to induce the artificial hatching of the eggs of *Neoechinorhynchus cylindratus*.

The author has increased the efficiency of Manter's method by several modifications. Instead of placing the eggs in a Syracuse watch glass to dry, a smear of eggs from the body cavity of a female worm, not more than one layer thick, is made on a clean glass slide, and allowed to become completely desiccated, either by drying in an incubator at 37.5° C for 3-4 hours or drying at room temperature overnight. The smears may then be stored in a covered slide-box until examined. In the humid Houston climate artificial hatching has been observed after storage for more than a month. However, the number of eggs that will hatch by this method decreases as the storage time is increased. In place of plain water for re-wetting the desiccated smears the author uses a dilute solution of neutral red (10 drops of a saturated solution in 20 cc of neutral distilled water). This re-wetting with the dilute vital stain induces artificial hatching just as well as plain water, and has the advantage of staining the larvae. A coverglass over the smear permits the use of the high dry and oil immersion objectives for the study of the acanthor stage. The central nuclear mass and the hypodermal nuclei of the acanthor stain a deep red. The presence of the stain in the tissue of the acanthor accentuates the outline of the subcuticular muscles and the retractor muscles of the rostellum. These muscles are invisible in unstained specimens.

The author has confirmed Manter's observation that only eggs from the body cavity of a mature female worm may be induced to hatch in this manner. Eggs from the feces of infected animals fail to hatch.

The author has been successful in hatching the eggs from the body cavities of the following Acanthocephala in this manner: *Moniliformis dubius*, *Macracanthorhynchus hirudinaceus*, *Macracanthorhynchus ingens*, *Hamanniella tortuosa*, and *Mediorhynchus grandis*. Attempts to hatch the eggs of *Neoechinorhynchus emydis* and *Centrorhynchus* sp. by this method have proved unsuccessful.—DONALD V. MOORE, Rice Institute, Houston, Texas.

NOTICE TO MEMBERS, SUBSCRIBERS, AND AUTHORS

The war effort must of necessity have its repercussions in the problem of publishing a scientific journal, especially one so exclusively dependent for its publication funds on income from members and subscribers, as is the case with the *JOURNAL OF PARASITOLOGY*. It was to be expected that foreign subscriptions would be reduced sharply in number, reductions which have been augmented by export prohibition to some countries. Individual energy needs to be used to compensate for these losses, not only by maintenance of membership in the Society, but by personal effort to increase domestic circulation. When inadequate resources are available for publishing, one effect is to postpone, in a disheartening way, the printing of acceptable articles.

During the year the Editorial Committee and Editorial Board have been examining possibilities which would tend to balance a net budget that is experiencing decrease both through lessened regular income and increased costs. They have decided to undertake, in experimental mood, two measures which will immediately strike the eye of the reader of Volume 29, and which will reduce to about 80 pages what requires 100 now. One of these is to use a slightly larger type page, holding the overall size of the *JOURNAL* page as at present. The other is to utilize more of the space, which for reasons of economy of effort in both the editorial office and the press, has hitherto been blank. Also to be mentioned is the omission of a separate index to the Supplement in this December issue. As the Supplement carries its own author index and the program is essentially a table of contents, the details of the compactly printed abstracts are considered readily accessible. These three methods of rationing of space will produce savings. Others may become necessary.

As a further contribution to the general problem, authors are urged to give a new quality of attention to the job of saying more in fewer words: shorter introductions, more compact factual presentations, briefer discussions, decreased overall lineage between the title and last bibliographic item. It would be a contribution to more than the war effort if articles of whatever length impressed the reader as being firm and trim, without excess wordiness, so that exceptions became conspicuous,—as are soft and flabby young men among the lean and well-muscled. To assist in such conditioning, authors of longer articles are to participate in greater degree in the sharing of cost of their publication. This is not to be interpreted, however, as qualifying the basic rule that "an article should meet primarily the standard of effective presentation of the material."

NORMAN R. STOLL,
Chairman, Editorial Committee

INDEX FOR VOLUME 28, NOS. 1-6

(For December Supplement consult its Author Index and Program.)

Acanthocephalan, new	385
Acanthor stage in acanthocephalan life histories, improved technique for study of	495
ACKERT, J. E. Natural resistance to helminthic infections	1
ACKERT, J. E. Portrait	Frontispiece
<i>Aedes aegypti</i> , a strain selected for susceptibility to <i>Plasmodium lophurae</i>	457
Alligator, strigeid trematodes of	51
America, North and South, studies on some Gordiacea of	213
American chiggers (Trombiculinae), taxonomy of some	485
AMERICAN SOCIETY OF PARASITOLOGISTS:	
17th annual meeting, Dallas, Texas, Dec. 29-31, 1941	247
Changes in membership	250
List of members	Supplement 35
Preliminary announcement, 18th annual meeting, New York City, Dec. 28-30, 1942; Postponement	432; 506
Report of the Committee on Terminology of Strains of Avian Malaria	250
<i>Anopheles</i> in Georgia, macroscopic species-identification of larval	299
Antelope, American, new coccidium from	167
<i>Aporina delafondi</i> (Railliet), anoplocephalid tapeworm, the mourning dove a new host for	495
<i>Aspidogaster conchicola</i> , observations on life history and taxonomic relationships of	467
Avian Malaria, report of A.S.P. Committee on Terminology of Strains of	250
Avian plasmodia, some mosquito hosts to	127
Avian schistosomes, new species from Wisconsin and Michigan	25
Baermann apparatus in recovery of <i>Haemonchus contortus</i> larvae, efficiency of ..	433
Bat flea from Panama, a new species of	361
Bat infestations in human dwellings in Brazil, association of tick, <i>Ornithodoros talaje</i> with	165
BELLAMY, R. EDWARD. Observations on the macroscopic species-identification of larval <i>Anopheles</i> in Georgia	299
BELTRAN, ENRIQUE AND LUIS VARGAS. The intravenous inoculation of sporozoites of <i>Plasmodium gallinaceum</i>	246
BRACKETT, STERLING. Five new species of avian schistosomes from Wisconsin and Michigan, with the life cycle of <i>Gigantobilharzia gyrauli</i> (Brackett, 1940)	25
Brazil, association of tick, <i>Ornithodoros talaje</i> , with bat infestations in human dwellings in	165
Bugs, reduviid, reactions of man to feeding of	43
Bugs, transmission of rabbit fibroma (Shope) by haematophagous	395
BUTLER, ROBERT L., JR. AND REED O. CHRISTENSON. A simple apparatus for determining the viability of embryonated helminth ova	131
BYRD, ELON E. AND ROBERT J. REIBER. Strigeid trematodes of the alligator, with remarks on the prostate gland and terminal portions of the genital ducts ...	51
CABLE, R. M. AND A. V. HUNNINEN. Studies on the life history of <i>Siphodera vinalewardsii</i> (Linton) (Trematoda: Cryptogonimidae)	407
Caribou, parasites of woodland	423
CARVALHO, JOSÉ CANDIDO M. Note on the association of the tick, <i>Ornithodoros talaje</i> (Guérin-Meneville), with bat infestations in human dwellings in Brazil	165

CARVALHO, JOSÉ CANDIDO M. Studies on some Gordiacea of North and South America	213
Cats, natural infection with <i>Centrocestus armatus</i>	285
Cattle, two parasites of, occurring in horse	245
<i>Centrocestus formosanus</i> (Nishigori, 1924), metacercaria and adult of	285
Cestode, new, from pintail duck	141
CHANDLER, ASA C. Helminths of tree squirrels in southeast Texas	135
CHANDLER, ASA C. <i>Mesocestoides manteri</i> n. sp. from a lynx, with notes on other North American species of <i>Mesocestoides</i>	227
CHANDLER, ASA C. The helminths of raccoons in east Texas	255
CHEN, H. T. The metacercaria and adult of <i>Centrocestus formosanus</i> (Nishigori, 1924), with notes on the natural infection of rats and cats with <i>C. armatus</i> (Tanabe, 1922)	285
Chicken, excystation of coccidial oöcysts of	426
Chickens, periodicity of oöcyst discharge in coccidial infection of	346
Chiggers (Trombiculinae), taxonomy of some American	485
CHRISTENSEN, N. O. (see Roth and Christensen)	245
CHRISTENSON, REED O. (see Butler and Christenson)	131
Coccidial infection of chickens, periodicity of oöcyst discharge in	346
Coccidial oöcysts of the chicken, excystation of	426
Coccidian parasite of aquatic oligochaete	424
Coccidium, <i>Eimeria antilocaprae</i> , from American antelope	167
<i>Cochlosoma rostratum</i> from the turkey	349
Cockroach, observations on <i>Plistophora kudo</i> , a microsporidian of	399
COOK, ARTHUR H. (see Goble and Cook)	451
<i>Cooperia oncophora</i> of cattle occurring in horse	245
Copper salts, organic, tests upon <i>Strongylus vulgaris</i>	168
CORT, W. W. Robert William Hegner (1880-1942)	175
CORT, W. W. (see Olivier and Cort)	75
<i>Cotylurus communis</i> (Hughes), test of life cycle described for	75
<i>Cryptocotyle lingua</i> (Creplin, 1825), life history experiments with	91
<i>Cryptocotyle lingua</i> , 7-year-old infection in winkle	350
CULBERTSON, JAMES T. AND WALTER R. KESSLER. Age resistance of mice to <i>Trypanosoma cruzi</i>	155
CULBERTSON, JAMES T. Active immunity in mice against <i>Trichinella spiralis</i> ..	197
CULBERTSON, JAMES T. Passive transfer of immunity to <i>Trichinella spiralis</i> in the rat ..	203
Cultivation of <i>Trichomonas foetus</i> , a new thermostabile medium for prolonged bacteria-free	428
Culture, growth of <i>E. histolytica</i> in, effect of sulfonamide compounds on	441
Culture of <i>Neoaplectana chresima</i> , a new nematode parasitic in insects	123
Culture of <i>Trichomonas vaginalis</i> , physiology of a bacteria-free	369
<i>Cyclura cornuta</i> , iguana, new nematode from	165
<i>Cysticercus fasciolaris</i> , age resistance of albino rat to	207
DEEDS, FLOYD AND JOHN O. THOMAS. Studies on phenothiazine. XI. The excretion of phenothiazine	363
Dermatitis-producing schistosomes of eastern Massachusetts	103
<i>Dero limosa</i> , oligochaete, a new coccidian parasite of	424
DINABURG, A. G. The efficiency of the Baermann apparatus in the recovery of larvae of <i>Haemonchus contortus</i>	433
Dove, mourning, new host for <i>Aporina delafondi</i>	495
Duck, pintail, new cestode from	141
<i>Echinostomum callawayensis</i> Barker, life cycle of	431
Ectoparasite resistance, vitamins in relation to	170
Editorial Committee, Chairman. Notice to members, subscribers, and authors ..	495

Eggs, nematode, buoyancy of	95
EMLÉN, J. T., JR. (see Spurlock and Emlén)	341
<i>Endamoeba histolytica</i> , duration of human infection with	93
<i>Endamoeba histolytica</i> , effect of sulfonamide compounds on growth of, in culture	441
ERICKSON, ARNOLD B. AND P. R. HIGHBY. Parasites of the woodland caribou ..	423
EVANS, T. C. (see Levin and Evans)	477
EWING, H. E. Remarks on the taxonomy of some American chiggers (Trombiculinae), including the descriptions of new genera and species	485
Excystation of coccidial oöcysts of the chicken	426
FINLAY, C. E. Carlos Finlay and yellow fever (communication)	172
FISCHTHAL, JACOB H. A <i>Paragordius</i> larva (Gordiaceae) in a trematode	167
FISCHTHAL, JACOB H. Three new species of <i>Phyllodistomum</i> (Trematoda: Gorgoderidae) from Michigan fishes	269
FISCHTHAL, JACOB H. <i>Triganodistomum hypentelii</i> n. sp. (Trematoda: Lissorchiidae) from the hog sucker, <i>Hypentelium nigricans</i> (Le Sueur)	389
Fishes, Illinois, two new species of Myxosporidia from	83
Fishes, Michigan, new species of <i>Phyllodistomum</i> from	269
Flea, bat, from Panama, a new species of	361
Flicker, red-shafted, <i>Hypodectes chapini</i> n. sp. (Acarina) from	341
Florida Industrial School, intestinal parasites in boys of the	169
FORBES, WILLIAM C. Helminths from the Norway rat in northeastern Ohio ..	431
FULLER, H. S. AND Q. M. GEIMAN. South American cutaneous leishmaniasis in experimental animals	429
GEIMAN, Q. M. (see Fuller and Geiman)	429
Genitalia, male, musculature of, of <i>Haemonchus contortus</i>	351
Georgia, macroscopic species-identification of larval <i>Anopheles</i> from	299
GIRTH, H. B. (see Glaser, McCoy and Girth)	123
GLASER, R. W., E. E. MCCOY AND H. B. GIRTH. The biology and culture of <i>Neoplectana chresima</i> , a new nematode parasitic in insects	123
GOBLE, FRANS C. AND ARTHUR H. COOK. Notes on nematodes from the lungs and frontal sinuses of New York fur-bearers	451
GOBLE, FRANS C. <i>Crenosoma sederi</i> n. sp. (Nematoda: Metastrongyloidea), a new lungworm from the skunk (<i>Mephitis nigra</i>)	381
Gordiaceae, a <i>Paragordius</i> larva in a trematode	167
Gordiaceae of North and South America	213
GREENFIELD, SYLVIA H. Age resistance of the albino rat to <i>Cysticercus fasciolaris</i> ..	207
Gregarines of <i>Tenebrio molitor</i> larvae, study of temperature effects on	233
Grouse, ruffed, new parasite records from	92
Gull, black-headed (<i>Larus ridibundus</i>), immunity reaction in, infected with <i>Maritrema oöcysta</i>	423
<i>Haemonchus contortus</i> , efficiency of Baermann apparatus in recovery of larvae of ..	433
<i>Haemonchus contortus</i> , musculature of male genitalia of	351
HARKEMA, REINARD. <i>Pharyngostomoides procyonis</i> n.g., n. sp. (Strigeida), a trematode from the raccoon in North Carolina and Texas	117
HARKEMA, REINARD. The mourning dove, a new host of the anoplocephalid tapeworm, <i>Aporina delafondi</i> (Railliet)	495
HARTWELL, WAYNE M. Conservation of scholarly journals (communication) ..	174
HAUSCHKA, T. S. AND M. I. PENNYPACKER. <i>Adelina deronis</i> n. sp., a new coccidian parasite of the aquatic oligochaete, <i>Dero limosa</i>	424
Hawk, Swainson's, new spiruroid nematode from	241
HAWKINS, PHILIP A. <i>Sigmodon hispidus hispidus</i> , a new host for the strobilocercus of <i>Taenia taeniaeformis</i>	94
HEGNER, ROBERT WILLIAM. (1880-1942)	175
Helminth ova, determining viability of embryonated	131

Helminthic infections, natural resistance to	1
Helminths from Norway rat in northeastern Ohio	431
Helminths of raccoons in east Texas	255
Helminths of tree squirrels in southeast Texas	135
HENDERSON, M. E. (see Threlkeld and Henderson)	351
HERBER, E. C. Life history studies on two trematodes of the subfamily Notocotylinæ	179
HERDE, KARL E. A new spiruroid nematode, <i>Thelazia buteonis</i> , from Swainson's hawk	241
HIGHBY, P. R. (see Erickson and Highby)	423
HINSHAW, W. R. (see McNeil and Hinshaw)	349
Hogs in New Orleans area, incidence of <i>T. spiralis</i> in	223
Horse, occurrence in, of two nematode species of cattle	245
Host, nutritional requirements of the fowl cestode as demonstrated by starva- tion of	319
HUIZINGA, HENRY. <i>Eimeria antilocaprae</i> , a new coccidium from the American antelope	167
HUNNINEN, A. V. (see Cable and Hunninen)	407
Hydrogen ion concentration, effect of, on population of a bacteria-free culture of <i>Trichomonas vaginalis</i>	369
<i>Hypentelium nigricans</i> (Le Sueur), hog sucker, a new trematode from	389
Identification of larval <i>Anopheles</i> in Georgia	299
Iguana, <i>Cyclura cornuta</i> , a new nematode from	165
Illinois, new species of Myxosporidia from fishes of	83
Immunity, active, in mice against <i>Trichinella spiralis</i>	197
Immunity reaction in gull infected with <i>Maritrema oocysta</i>	423
Immunity to <i>Trichinella spiralis</i> in rat, passive transfer of	203
Insects, new nematode parasitic in	123
Intestinal parasites in boys	169
Intestinal protozoa, duration of human infection with	93
Intravenous inoculation of sporozoites of <i>Plasmodium gallinaceum</i>	246
ISHII, NOBUTARO. New parasite records from the ruffed grouse	92
JOHNSON, GARTH. Physiology of a bacteria-free culture of <i>Trichomonas</i> <i>vaginalis</i> . IV. Effect of hydrogen ion concentration and oxygen tension on population	369
Journals, conservation of scholarly	174
KARTMAN, LEO. A note on vitamins in relation to ectoparasite resistance	170
KESSLER, WALTER R. (see Culbertson and Kessler)	155
KIRBY, HAROLD. A parasite of the macronucleus of <i>Vorticella</i>	311
KIRSNER, JOSEPH B. (see Rodaniche and Kirsner)	441
KOHL, GLEN M. Siphonaptera: <i>Ptilopsylla dunni</i> , a new species of bat flea from Panama	361
<i>Larus ridibundus</i> L., black-headed gull, infected with <i>Maritrema oocysta</i> , im- munity reaction in	423
Leishmaniasis, South American cutaneous, in experimental animals	429
LEVIN, A. J. AND T. C. EVANS. The use of roentgen radiation in locating an origin of host resistance to <i>Trichinella spiralis</i> infections	477
LEVINE, P. P. Excystation of coccidial oöcysts of the chicken	426
LEVINE, P. P. The periodicity of oöcyst discharge in coccidial infection of chickens	346
Life cycle of <i>Cotylurus communis</i> (Hughes)	75
Life cycle of <i>Echinostomum callawayensis</i> Barker	431
Life cycle of <i>Gigantobilharzia gyrauli</i> (Brackett, 1940)	25

Life histories, acanthocephalan, improved technique for study of acanthor stage in	495
Life history experiments with <i>Cryptocotyle lingua</i> (Creplin, 1825)	91
Life history of <i>Aspidogaster conchicola</i>	467
Life history of <i>Porocephalus crotali</i>	277
Life history of <i>Siphodera vinaldwadsii</i> (Linton)	407
Life history studies on two trematodes (Notocotylinae)	179
<i>Littorina littorea</i> L., winkle, 7-year-old infection of <i>Cryptocotyle lingua</i> in	350
Lizard, <i>Tupinambis teguixin</i> , from South America, <i>Hemogregarina triatomae</i> n. sp. from	147
Louisiana muskrat, life history of <i>Porocephalus crotali</i> from	277
Louisiana muskrats, parasitological survey of	348
Lungs, nematodes from	451
Lungworm, new, from the skunk	381
Lynx, new cestode from	227
MACDOUGALL, MARY MURIEL. A study of temperature effects on gregarines of <i>Tenebrio molitor</i> larvae	233
<i>Macracanthorhynchus hirudinaceus</i> , chemistry of	315
MACY, RALPH W. The life cycle of the trematode <i>Echinostomum callawayensis</i> Barker	431
Malaria, a strain of <i>Aedes aegypti</i> selected for susceptibility to <i>Plasmodium</i> <i>lophurae</i>	457
Malaria, report of A.S.P. Committee on Terminology of Strains of Avian	250
Man, association of tick, <i>Ornithodoros talaje</i> , with bat infestations in dwellings of	165
Man, duration of infection with intestinal protozoa	93
Man, intestinal parasites in boys of the Florida Industrial School	169
Man, reactions to feeding of reduviid bugs	43
<i>Maritrema oocysta</i> Lebour, 1907, on immunity reaction in gull infected with	423
Massachusetts, studies on dermatitis-producing schistosomes in eastern	103
McCoy, E. E. (see Glaser, McCoy and Girth)	123
McNEIL, E. AND W. R. HINSHAW. <i>Cochlosoma rostratum</i> from the turkey	349
MEGLITSCH, PAUL A. On two new species of Myxosporidia from Illinois fishes	83
MELENEY, HENRY E. The duration of human infection with <i>Endamoeba histo-</i> <i>lytica</i> and other intestinal protozoa	93
<i>Mephitis nigra</i> , skunk, a new lungworm from	381
<i>Mesocestoides</i> , new species and notes on North American species of	227
Mice, active immunity against <i>Trichinella spiralis</i>	197
Mice, age resistance of, to <i>Trypanosoma cruzi</i>	155
Mice, device for administering particulate material to	92
Michigan fishes, new species of <i>Phyllodistomum</i> from	269
Michigan, new species of avian schistosomes from	25
Microsporidian of cockroach, observations on <i>Plistophora kudo</i>	399
MOHAN, BADRI NATH (see Russell and Mohan)	127
MOORE, DONALD V. An improved technique for the study of the acanthor stage in certain acanthocephalan life histories	495
Mosquito hosts to avian plasmodia	127
Musculature of male genitalia of <i>Haemonchus contortus</i>	351
Muskrats, Louisiana, parasitological survey of	348
Myxosporidia, two new species from Illinois fishes	83
Nematode eggs, buoyancy of	95
Nematodes from lungs and frontal sinuses of New York fur-bearers	451
<i>Neoaplectana chresima</i> , new nematode parasitic in insects, biology and culture of	123
<i>Neotoma</i> , new <i>Nematodirus</i> from	159
New genera (indicated *) and new species (volume 28, 1942):	
* <i>Acomatacarus arizonensis</i> Ewing	490
<i>Adelina deronis</i> Hauschka and Pennypacker	424

<i>Chloromyxum opladeli</i> Meglitsch	83
<i>Chloromyxum thompsoni</i> Meglitsch	88
* <i>Comatacarus</i> Ewing	489
<i>Comatacarus americanus</i> Ewing	490
<i>Comatacarus occidentalis</i> Ewing	489
<i>Crenosoma zedleri</i> Goble	381
<i>Dirofilaria tenuis</i> Chandler	262
<i>Eimeria antilocaprae</i> Huizinga	168
<i>Filicollis altmani</i> Perry	386
<i>Gigantobilharsia lawayi</i> Brackett	33
<i>Gnathostoma procyonis</i> Chandler	260
<i>Haemogregarina triatomae</i> Osimani	152
<i>Hymenolepis mastigopraedita</i> Polk	141
<i>Hypodectes chapini</i> Spurlock and Emlen	342
<i>Mesocestoides manteri</i> Chandler	229
<i>Microfilaria alpha</i> Chandler	139
<i>Microfilaria beta</i> Chandler	139
<i>Molineus barbatus</i> Chandler	259
<i>Nematodirus tortuosus</i> Tucker	159
<i>Neoplectana chresima</i> Steiner	123
* <i>Neochordodes</i> Carvalho	215
<i>Neochordodes moraisi</i> Carvalho	219
<i>Notocotylus stagnicola</i> Herber	188
<i>Oochoristica procyonis</i> Chandler	256
<i>Paragordius esavianus</i> Carvalho	218
<i>Paralaeuris cuckleri</i> Walton	166
* <i>Pharyngostomoides procyonis</i> Harkema	117
<i>Phyllodistomum etheostomae</i> Fischthal	Supplement, Abstract 28
<i>Phyllodistomum nocomis</i> Fischthal	273
<i>Phyllodistomum notropidis</i> Fischthal	271
<i>Phyllodistomum semotili</i> Fischthal	269
<i>Pseudobilharziella burnetti</i> Brackett	29
<i>Pseudobilharziella horiconensis</i> Brackett	29
<i>Pseudobilharziella kegonsensis</i> Brackett	28
<i>Pseudobilharziella waubesensis</i> Brackett	27
* <i>Pseudochordodes</i> Carvalho	216
<i>Pseudochordodes manteri</i> Carvalho	217
* <i>Pseudocrocodilicola americanense</i> Byrd and Reiber	55
<i>Pseudocrocodilicola georgiana</i> Byrd and Reiber	56
<i>Pseudoneodiplostomum acetabulata</i> Byrd and Reiber	51
<i>Ptilopsylla dunni</i> Kohls	361
<i>Raillietina</i> (<i>Raillietina</i>) <i>bakeri</i> Chandler	136
<i>Schöngastia lynni</i> Ewing	487
<i>Stempellia moniezi</i> Jones	Supplement, Abstract 5
<i>Strongyloides robustus</i> Chandler	138
<i>Synhimantus longigutturata</i> Chandler	260
<i>Thelazia buteonis</i> Herde	241
<i>Triganodistomum hypentelii</i> Fischthal	389
<i>Trombicula californica</i> Ewing	488
<i>Trombicula rokhveri</i> Ewing	488
<i>Walchia americana</i> Ewing	491
New Orleans area, incidence of <i>Trichinella spiralis</i> in hogs and rats of	223
New York fur-bearers, nematodes from lungs and frontal sinuses of	451
North Carolina, new trematode from raccoon in	117
Notocotylinae, trematode subfamily, two life history studies of	179
Nutritional requirements of the fowl cestode, <i>Raillietina cesticillus</i>	319

Ohio, helminths from Norway rat in northeastern	431
Oligochaete, <i>Dero limosa</i> , a new coccidian parasite of the aquatic	424
OLIVIER, LOUIS and W. W. CORT. An experimental test of the life cycle described for <i>Cotylurus communis</i> (Hughes)	75
<i>Ornithodoros talaje</i> , tick, associated with bat infestations in human dwellings ..	165
OSIMANI, J. J. <i>Haemogregarina triatomae</i> n. sp. from a South American lizard <i>Tupinambis teguixin</i> transmitted by the reduviid <i>Triatoma rubrovaria</i>	147
<i>Ostertagia ostertagi</i> of cattle occurring in horse	245
Ova, helminth, determining viability of embryonated	131
Ova, helminth, in formalinized feces, recovery of	345
Oxygen tension, effect of, on population of a bacteria-free culture of <i>Trichomonas vaginalis</i>	369
Panama, new species of bat flea from	361
<i>Paragordius</i> larva in a trematode	167
Parasites, intestinal, in boys	169
Parasites of the woodland caribou	423
PENN, GEORGE H., JR. Parasitological survey of Louisiana muskrats	348
PENN, GEORGE H., JR. The life history of <i>Porocephalus crotali</i> , a parasite of the Louisiana muskrat	277
PENNER, LAWRENCE R. Studies on dermatitis-producing schistosomes in eastern Massachusetts, with emphasis on the status of <i>Schistosomatum pathlocopticum</i> Tanabe, 1923	103
PENNYPACKER, M. I. (see Hauschka and Pennypacker)	424
PERES, CHARLES E. <i>Trichinella spiralis</i> . II. Incidence of infection in hogs and rats in the New Orleans area	223
Periodicity of oöcyst discharge in coccidial infection of chickens	346
PERRY, MARY LOUISE. A new species of the acanthocephalan genus <i>Filicollis</i> ..	385
Phenothiazine, studies on: excretion of phenothiazone	363
PHILIP, CORNELIUS B. Mechanical transmission of rabbit fibroma (Shope) by certain haematophagous bugs	395
<i>Plasmodium cathemerium</i> , oral transmission of, by means of tissues	245
<i>Plasmodium gallinaceum</i> , intravenous inoculation of sporozoites of	246
<i>Plasmodium gallinaceum</i> , mosquito hosts of	127
<i>Plasmodium lophurae</i> , avian malaria parasite, strain of <i>Aedes aegypti</i> selected for susceptibility to	457
<i>Plistophora kudoi</i> , microsporidian of cockroach, observations on	399
POLK, SEIGUL J. <i>Hymenolepis mastigopraedita</i> , a new cestode from a pintail duck	141
<i>Porocephalus crotali</i> , from Louisiana muskrat, life history of	277
Protozoa, intestinal, duration of human infection with	93
Raccoon in North Carolina and Texas, new trematode from	117
Raccoons in east Texas, helminths of	255
<i>Raillietina cesticillus</i> (Molin), nutritional requirements of	319
RAMSEY, JUANITA (see Sprague and Ramsey)	399
Rat, albino, age resistance of, to <i>Cysticercus fasciolaris</i>	207
Rat, <i>Neotoma</i> , new <i>Nematodirus</i> from	159
Rat, Norway, helminths from, in northeastern Ohio	431
Rat, passive transfer of immunity to <i>Trichinella spiralis</i> in the	203
Rats, natural infection with <i>Centrocestus armatus</i>	285
Rats of New Orleans area, incidence of <i>T. spiralis</i> in	223
Reduviid bugs, reactions of man to feeding of	43
Reduviid, <i>Triatoma rubrovaria</i> , transmitter of hemogregarine of lizard	147
REIBER, ROBERT J. (see Byrd and Reiber)	51
REID, W. M. Certain nutritional requirements of the fowl cestode <i>Raillietina cesticillus</i> (Molin) as demonstrated by short periods of starvation of the host ..	319

Resistance, age, of albino rat to <i>Cysticercus fasciolaris</i>	207
Resistance, age, of mice to <i>Trypanosoma cruzi</i>	155
Resistance, host, to <i>Trichinella spiralis</i> , use of roentgen radiation in locating origin of	477
Resistance to helminthic infections, natural	1
Resistance, vitamins in relation to ectoparasite	170
RODANICHE, ENID C. AND JOSEPH B. KIRSNER. The effect of sulfonamide compounds on the growth of <i>Endamoeba histolytica</i> in culture	441
Roentgen radiation, use in locating origin of host resistance to <i>Trichinella spiralis</i>	477
ROTH, HANS AND N. O. CHRISTENSEN. Occurrence in the horse of two parasites of cattle, <i>Ostertagia ostertagi</i> (Stiles, 1892) and <i>Cooperia oncophora</i> (Railliet, 1898)	245
ROTHSCHILD, MIRIAM. A further note on life history experiments with <i>Cryptocotyle lingua</i> (Creplin, 1825)	91
ROTHSCHILD, MIRIAM. A note on immunity reaction in the black-headed gull (<i>Larus ridibundus</i> L.) infected with <i>Maritrema oocysta</i> Lebour, 1907	423
ROTHSCHILD, MIRIAM. A seven-year-old infection of <i>Cryptocotyle lingua</i> Creplin in the wrinkle <i>Littorina littorea</i> L.	350
RUSSELL, PAUL F. AND BADRI NATH MOHAN. Some mosquito hosts to avian plasmodia with special reference to <i>Plasmodium gallinaceum</i>	127
SAURWEIN, JEAN (see von Brand and Saurwein)	315
SAWITZ, WILLI. The buoyancy of certain nematode eggs	95
<i>Schistosomatum pathlocorticum</i> Tanabe, 1923, studies on	103
Schistosomes, avian, from Wisconsin and Michigan	25
Schistosomes, dermatitis-producing, studies on	103
SCHNEIDER, MORRIS D. A new thermostable medium for the prolonged bacteria-free cultivation of <i>Trichomonas foetus</i>	428
<i>Sigmodon h. hispidus</i> , new host for strobilocercus of <i>Taenia taeniaeformis</i>	94
Sinuses, frontal, nematodes from	451
<i>Siphodera vinalledwardsii</i> , studies on life history of	407
Skunk, <i>Mephitis nigra</i> , a new lungworm from	381
South America, <i>Hemogregarina triatomae</i> n. sp. from lizard, <i>Tupinambis teguixin</i> , of	147
South American cutaneous leishmaniasis in experimental animals	429
SPRAGUE, VICTOR AND JUANITA RAMSEY. Further observations on <i>Plistophora kudoii</i> , a microsporidian of the cockroach	399
SPURLOCK, G. M. AND J. T. EMLEN, JR. <i>Hypodectes chapini</i> n. sp. (Acarina) from the red-shafted flicker	341
Squirrels, tree, helminths of, in southeast Texas	135
Strains of Avian Malaria, report of A.S.P. Committee on Terminology of	250
<i>Strongylus vulgaris</i> , tests with organic copper salts upon	168
Sucker, hog, <i>Hypentelium nigricans</i> , a new trematode, <i>Triganodistomum hypentelii</i> , from	389
Sulfonamide compounds, effect on growth of <i>E. histolytica</i> in culture	441
SUMMERS, WILLIAM A. A modification of zinc sulfate centrifugal flotation method for recovery of helminth ova in formalinized feces	345
SUMMERS, WILLIAM A. Intestinal parasites in boys of the Florida Industrial School	169
Survey, parasitological, of Louisiana muskrats	348
Susceptibility to <i>Plasmodium lophurae</i> , a strain of <i>Aedes aegypti</i> selected for	457
<i>Taenia taeniaeformis</i> , strobilocercus of, <i>Sigmodon h. hispidus</i> a new host for	94
Temperature effects on gregarines of <i>Tenebrio molitor</i> larvae	233
Terminology of Strains of Avian Malaria, report of A.S.P. Committee on	250
Texas, east, helminths of raccoons in	255
Texas, new trematode of raccoon in	117

Texas, southeast, helminths of tree squirrels in	135
THOMAS, JOHN O. (see DeEds and Thomas)	363
THRELKELD, W. L. AND M. E. HENDERSON. Notes on the musculature of the male genitalia of <i>Haemonchus contortus</i>	351
Tick, <i>Ornithodoros talaje</i> , associated with bat infestations in human dwellings	165
TRAGER, WILLIAM. A strain of the mosquito <i>Aedes aegypti</i> selected for susceptibility to the avian parasite <i>Plasmodium lophurae</i>	457
Transmission of rabbit fibroma (Shope) by certain hematophagous bugs	395
Transmission, oral, of <i>Plasmodium cathemerium</i> by means of tissues	245
Trematode, new, from raccoon in North Carolina and Texas	117
Trematode, <i>Paragordius</i> larva in a	167
Trematodes, life history studies on two Notocotylineae	179
Trematodes, strigeid, of the alligator	51
<i>Triatoma rubrovaria</i> , reduviid transmitting hemogregarine of lizard	147
<i>Trichinella spiralis</i> , active immunity in mice against	197
<i>Trichinella spiralis</i> , incidence in hogs and rats in New Orleans area	223
<i>Trichinella spiralis</i> in the rat, passive transfer of immunity to	203
<i>Trichinella spiralis</i> , use of roentgen radiation in locating origin of host resistance to	477
<i>Trichomonas foetus</i> , a new medium for prolonged bacteria-free cultivation of ...	428
Trombiculinae, American chiggers, taxonomy of some	485
<i>Trypanosoma cruzi</i> , age resistance of mice to	155
TUCKER, HAL. <i>Nematodirus tortuosus</i> n. sp. (Nematoda) from the rat, <i>Neotoma</i>	159
<i>Tupinambis teguixin</i> , South American lizard, <i>Hemogregarina triatomae</i> n. sp. from, transmitted by <i>Triatoma rubrovaria</i>	147
Turkey, <i>Cochlosoma rostratum</i> from the	349
VARGAS, LUIS (see Beltran and Vargas)	246
Viability of embryonated helminth ova	131
Vitamins in relation to ectoparasite resistance	170
VON BRAND, THEODOR AND JEAN SAURWEIN. Further studies upon the chemistry of <i>Macracanthorhynchus hirudinaceus</i>	315
<i>Vorticella</i> , a parasite of macronucleus of	311
WALLACE, F. G. A device for administering particulate material to mice	92
WALTON, A. C. <i>Paralaeuris cuckleri</i> n. sp. (Nematoda) from the iguana (<i>Cyclura cornuta</i>)	165
WHITLOCK, J. H. Studies upon <i>Strongylus vulgaris</i> . VI. Tests with organic copper salts	168
WILLIAMS, CHARLES O. Observations on the life history and taxonomic relationships of the trematode <i>Aspidogaster conchicola</i>	467
Winkle, <i>Littorina littorea</i> , 7-year-old infection of <i>Cryptocotyle lingua</i> in	350
Wisconsin, new species of avian schistosomes from	25
WOLFSON, FRUMA. Oral transmission of <i>Plasmodium cathemerium</i> by means of tissues	245
WOOD, SHERWIN F. Reactions of man to the feeding of reduviid bugs	43
Yellow fever, Carlos Finlay and	172
Zinc sulfate centrifugal flotation modification for recovery of helminth ova in formolinized feces	345

AMERICAN SOCIETY OF PARASITOLOGISTS

Announcement of Postponement of the New York Meeting

On November 28, a letter was received from Dr. F. R. Moulton, Permanent Secretary, stating that the Executive Committee of the American Association for the Advancement of Science had voted to postpone the New York meeting, "in compliance with the request of the Office of Defense Transportation. . . . As yet no plans have been formulated for holding the meeting in the immediate future."

By vote of Council of the American Society of Parasitologists, the 18th Annual Meeting of the Society, scheduled to be held with the New York meeting of the A. A. S., was in turn postponed.

JAMES T. CULBERTSON,
Secretary

December 1, 1942.